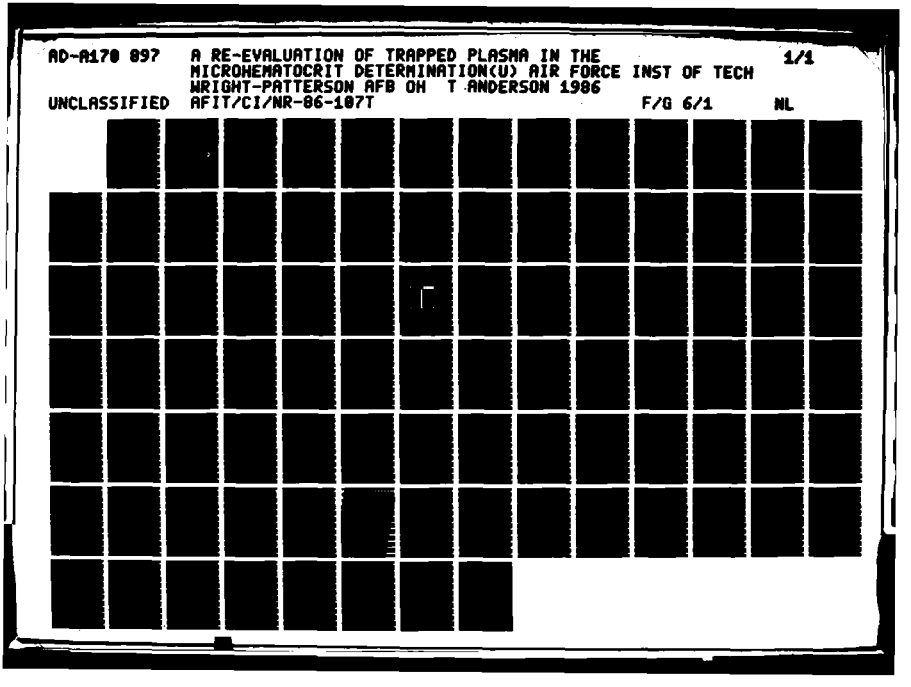
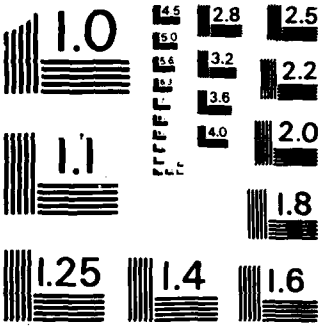

AD-A170 897 A RE-EVALUATION OF TRAPPED PLASMA IN THE MICROHEMATOCRIT DETERMINATION(U) AIR FORCE INST OF TECH WRIGHT-PATTERSON AFB OH T ANDERSON 1986
1/1
UNCLASSIFIED AFIT/CI/NR-86-107T
F/G 6/1
NL



AD-A170 897

ata Entered)

N PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
AFIT/CI/NR 86-107T	2. GOVT ACCESSION NO. AD-A170 897 — 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) A Re-evaluation of Trapped Plasma in the Microhematocrit Determination	5. TYPE OF REPORT & PERIOD COVERED THESIS/DISSERTATION
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Timothy Andersen	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS AFIT STUDENT AT: University of Washington	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS AFIT/NR WPAFB OH 45433-6583	12. REPORT DATE 1986
	13. NUMBER OF PAGES 80
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) UNCLAS
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

DTIC ELECTE AUG 13 1986 S D B

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

APPROVED FOR PUBLIC RELEASE: IAW AFR 190-1

LYNN E. WOLAVER 6 AUG 86
Dean for Research and Professional Development
AFIT/NR

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

ATTACHED.

86 8 12 045

D FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

A Re-evaluation of Trapped Plasma in the Microhematocrit Determination

by

TIMOTHY ANDERSEN

A thesis submitted in partial fulfillment
of the requirements for the degree of

Master of Science

University of Washington

1986

Approved by _____
(Chairman of Supervisory Committee)

_____

_____

Program Authorized
to Offer Degree Department of Laboratory Medicine

Date May 23, 1986

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at the University of Washington, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Any other reproduction for any purposes or by any means shall not be allowed without my written permission.

Signature Timothy Andersen

Date 23 May 86

TABLE OF CONTENTS

LIST OF TABLES

Number Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Meaning
cpm	counts per minute
x g	x gravity
$^{131}_{I}$ HSA	^{131}Iodinated human serum albumin
MCHC	mean cell hemoglobin concentration
MCV	mean cell volume
m/s^2	meters/second^2
pcv	packed cell volume
RCF	relative centrifugal force
RDW	red cell distribution width calculation
tp	total trapped plasma

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. Detter for his assistance in my research and manuscript preparation. In addition, I would like to thank Jan Stover for her technical help throughout the project. Finally, a special thanks to my wife for her patience and understanding.

CHAPTER ONE

INTRODUCTION

Introductory Remarks

Interest in the subject of trapped plasma seems to peak about every ten years, at least as demonstrated by the number of articles published. By no means has this concern been resolved. I will begin with a definition of trapped plasma. From there I will introduce you to certain historical remarks regarding the centrifuged hematocrit and the variation produced by trapped plasma. Finally, I will close the introduction with a summary of the major works on this subject and state the project synopsis.

Trapped Plasma

Trapped plasma is that amount of intracellular plasma remaining in the packed cell column of the microhematocrit after centrifugation. It artificially increases the packed cell column constituting a positive bias to the hematocrit. The amount of trapped plasma varies as a function of cell packing. Foremost are the physical forces applied in the microhematocrit determination. The speed of the centrifuge and length

of time of centrifugation are the major contributors. Cell packing is also dependent on the deformability of the cell, an intrinsic property of the erythrocyte. To a lesser extent, trapped plasma is affected by factors such as the anticoagulant used and plasma osmolality (1).

The Hematocrit

The hematocrit was first introduced by Hedin in 1890 (cited in 2, 10). It is now one of the most frequently requested lab procedures. The hematocrit is the ratio of the volume of erythrocytes to that of whole blood (11). It is expressed as a percentage or a decimal fraction. The hematocrit is of fundamental importance to the clinician in assessing the patient's blood volume and total erythrocyte mass. Both of these vital measurements are estimated from the hematocrit value, since their direct measurement is relatively difficult. Additionally, two of the erythrocyte indices (MCV,MCHC) are derived from the hematocrit.

Trapped plasma may cause significant error in the measurement of the hematocrit. Trapped plasma as high as 20% of the packed cell column has been reported (2). If the trapped plasma were 20%, a hematocrit of 30% would

be artificially inflated by 6%, resulting in a true hematocrit of 24%. Even small variations of the hematocrit caused by trapped plasma can prove to be critical. For example, the hematocrit is an essential index in the evaluation of neonatal hyperviscosity syndrome which is characterized by hypoglycemia, congestive heart failure, seizures, and neurologic sequelae (4). Hematocrits from healthy neonates range from 51% to 65% (3). Hyperviscosity is a distinct risk at a hematocrit above 60% and partial exchange transfusion is recommended above 65%. With partial exchange transfusion recommended at a hematocrit barely above the reference range, there is little room for error. Yet Penn, et al in 1978 (4) reported that trapped plasma can increase a neonatal hematocrit of 65% to as high as 67% or 68%.

The centrifuged microhematocrit, the most widely used method, is now being supplanted by the derived hematocrits generated by electronic particle counters. However, due to financial constraints and convenience many labs in the United States still retain the manually performed microhematocrit. One of the advantages of the electronic particle counter is that the hematocrit is derived directly from cell size measurements and the red cell count. (Hct = MCV x RBC count / 10) Since centrifugation is avoided there isn't any trapped plasma

error. Nevertheless, cell volume measurements on these counters must be carefully calibrated. These calibrations are based on hematocrit determinations. This raises an additional issue. If some plasma is trapped in any hematocrit determination, should the reference material used to calibrate the electronic particle counter be corrected? At one time all Coulter calibrators were arbitrarily corrected for 3% trapped plasma. This practice has subsequently been discontinued. Since there is a potential difference in the hematocrit value depending on which determination method is used, it is important to establish the magnitude of the differences in conditions which might be expected to cause variation.

Literature Review of Trapped Plasma

Chaplin and Mollison in 1952 were the first to fully investigate the amount of trapped plasma under various conditions (5). Using the hematocrit technique described by Wintrobe and a low speed centrifuge, they concluded that the trapped plasma of normal blood is about 2.5%. With this technique, 100 mm glass tubes were filled with anticoagulated blood. The tube was capped to prevent evaporation and centrifuged for 30 minutes at 1,500 xg (11). Recognize that standard

centrifuges in most laboratories during that time were incapable of maintaining speeds much above 3,000 rpm. Chaplin and Mollison showed that the trapped plasma varies as a function of cell packing and the latter was related to the speed and length of time of centrifugation. Additionally, they demonstrated trapped plasma correlated directly to the packed cell volume due to a corresponding reduction of the effective radius of centrifugation at higher hematocrit values. More recent research utilized higher speed centrifuges. Garby and Vuille in 1961 (6) evaluated trapped plasma using capillary tubes centrifuged at 10,000 xg for ten minutes. They determined the average trapped plasma for blood from healthy subjects is 1.31%. However, their study group was limited to five subjects. England, Walford and Waters' report in 1971 (2) was also based on a high speed centrifuge, the Hawksley Micro-Haematocrit Centrifuge. Spinning for five minutes at 12,000 xg, they concluded the trapped plasma reference value is 3.22%, based on twenty-seven subjects. They further evaluated trapped plasma in specific red cell diseases and stated that trapped plasma "values of 5-6% may be found in hypochromic anemias, and even over 20% in sickled samples of blood." Because of the error introduced by trapped plasma, England, et al advocated calibrating the Coulter S to read a true hematocrit.

This is accomplished by setting the Coulter to read 1 1/2 units less than the centrifugation value of the reference material distributed by Coulter Electronics. The most recent comprehensive examination of trapped plasma is by Pearson and Guthrie in 1982 (7). They concluded that the 3% estimate by England, et al is too high. Using essentially the same technique, but centrifuging for ten minutes at 13,000 xg, they found an average trapped plasma of 1.53% in twenty-five healthy subjects. Pearson and Guthrie demonstrated marginally higher values in certain red cell disorders. Statistically significant differences were found in patients with iron deficient anemia (trapped plasma value of 1.82%) and in patients with HbS (trapped plasma values of 1.68% for heterozygous and 1.70% for homozygous conditions). Pearson and Guthrie recommend using a trapped plasma value of 1.5% instead of 3% for control material issued by Coulter Electronics.

The means for the reference values of these four previous studies are listed below:

Chaplin and Mollison (1952)	2.5%
Garby and Vuille (1961)	1.3%
England, Walford, and Waters (1972)	3.2%
Pearson and Guthrie (1982)	1.5%

Other investigators have looked at the trapped plasma in specific red cell disorders. Furth in 1956 (8) reported that trapped plasma values were increased in samples of healthy subjects with hereditary spherocytosis. Economou-Marvou and Tsenghi in 1965 (9) showed that trapped plasma levels in samples from children with severe thalassemia were higher than in samples from healthy children.

Thesis Synopsis

In this thesis I will re-examine the question of trapped plasma values using a different microhematocrit determination method. Though distinctly different from all earlier mentioned sources, this method is commonly employed by laboratories in the United States. Accordingly, I will establish a new trapped plasma reference range and re-assess selected red cell disorders. Finally, I will determine what effect hematocrit level has on trapped plasma. I will examine hematocrits artificially created by addition or subtraction of plasma from the specimen.

CHAPTER TWO

METHODS AND MATERIALS

Microhematocrit Determination

One of the major goals of this project is to ascertain whether the microhematocrit method used in this study produces a trapped plasma value which is different from previously reported sources (2,5-7). All specimens in my study were collected in tubes containing K_3EDTA anticoagulant. Two drops of radiolabeled human serum albumin per mL of whole blood were added for the trapped plasma determination technique (See below ^{131}I Human Serum Albumin). The blood was mixed for at least three minutes and then drawn into nonheparinized, glass capillary tubes (Dade Diagnostics, McGaw Park, IL 60085) to a length of 60-70 mm. Triplicate capillary tubes were prepared for every determination. The tubes were then plugged at one end with Seal-Ease clay (Clay-Adams, Parsippany, NJ 07054). Capillary tubes with uneven blood-to-clay interfaces were discarded. The tube ends that had been dipped into the radiolabeled blood were wiped clean with a 5% (v/v) solution of Atom Clean (Diagnostic Imaging Supply Co., Berkley, MI) to remove any exterior contamination. Ten empty capillary tubes were plugged with clay and then dipped into radiolabeled blood. This procedure results in

radiolabeled blood only on the outside of the capillary tube which was wiped with Atom Clean. Remaining radiation was then counted on the gamma counter. Tubes with counts $\leq$ 25 cpm are considered uncontaminated.

Tube	cpm	Tube	cpm
1	4	6	67*
2	7	7	0
3	17	8	24
4	4	9	0
5	5	10	25

*Exposed end of clay plug was not blotted with Atom Clean.

The tubes were centrifuged for five minutes on an IEC MB Microhematocrit Centrifuge (Damon Industries, Needham Hts, MA 02194). Hematocrit values were determined by measuring the capillary tubes on a Micro-Capillary Reader (Damon) to the nearest 0.1%. Throughout the project hematocrits were independently read by a second person. At no time did the two readings disagree by 2% or more.

Relative Centrifugal Force

The Relative Centrifugal Force at the red cell meniscus of the IEC MB Centrifuge used in this project is approximately 10,500 x g. The RCF in m/s^2 is given by the formula:

$$1.1 \times 10^{-5} \times R \times N^2$$

where 1.1×10^{-5} is a constant, R is the centrifugal radius (in mm) from the red cell meniscus to the axis of rotation, and N is the number of revolutions per minute (12). The centrifugal radius for a 65 mm column of blood with a hematocrit of 40% was calculated at 64.6 mm. The radius from centrifuge center to outer rim was measured at 90.6 mm. Subtracting the length beneath the red cell meniscus from the centrifuge rim, we have:

$$90.6 \text{ mm} - (65 \text{ mm} \times .4) = 64.6 \text{ mm}$$

The centrifuge speed was measured at 12,000 rpm $\pm$ 200 and verified with a stroboscope at several intervals over the course of the project. Using these variables and dividing the entire quantity by the standard acceleration of gravity (9.807 m/s^2) we have:

$$(1.1 \times 10^{-5} \times 64.6 \times 12{,}000^2) / 9.807 = 10{,}434 \times g$$

Optimum Packing Time

Five minutes of centrifugation was determined to be the optimum packing time based on the following study. Eighteen replicates of a single sample of healthy blood

were centrifuged for each of two, four, five, six, eight, and ten minute intervals. Mean hematocrit and relative trapped plasma values were computed for each time interval. These mean hematocrit and relative trapped plasma values were then plotted against the various time intervals (See Figures 1 and 2). The optimum packing time is the minimum time needed for maximum cell packing at a particular RCF (13). This endpoint is illustrated by the hematocrit. In this study hematocrit values do not decrease if centrifugation is continued beyond five minutes. Note that the relative trapped plasma values correspond to the hematocrit values at each time interval, but are slightly more variable.

Breaking Capillary Tubes

After the hematocrit was read, the capillary tubes were scored with a metal file and broken just below the red cell meniscus. The two resulting segments are hereafter called the packed cell volume and plasma segments. Attempts to break the capillary tubes exactly at the meniscus resulted in small amounts of plasma from the plasma segment to separate with the packed cell volume segment. This doubled or even tripled the trapped plasma value measurement. By breaking below the red cell meniscus, approximately one millimeter of packed cells was

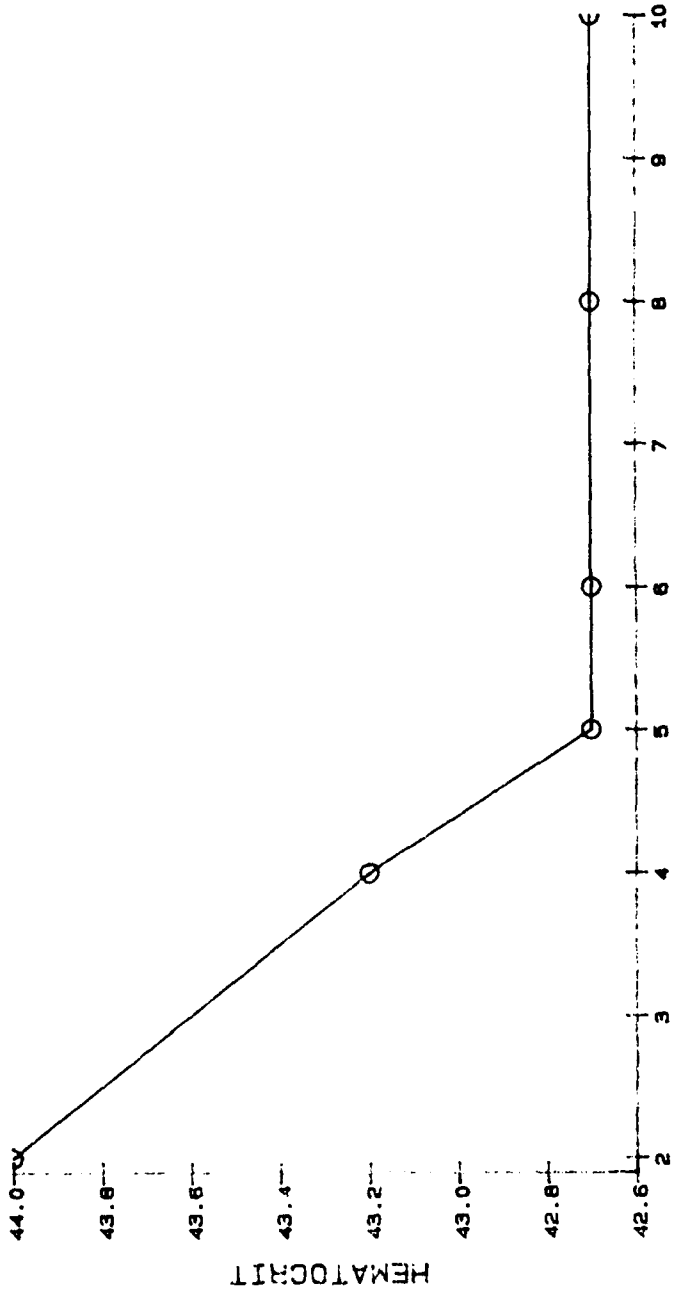


Figure 1. Optimum Packing Time (Hematocrit)

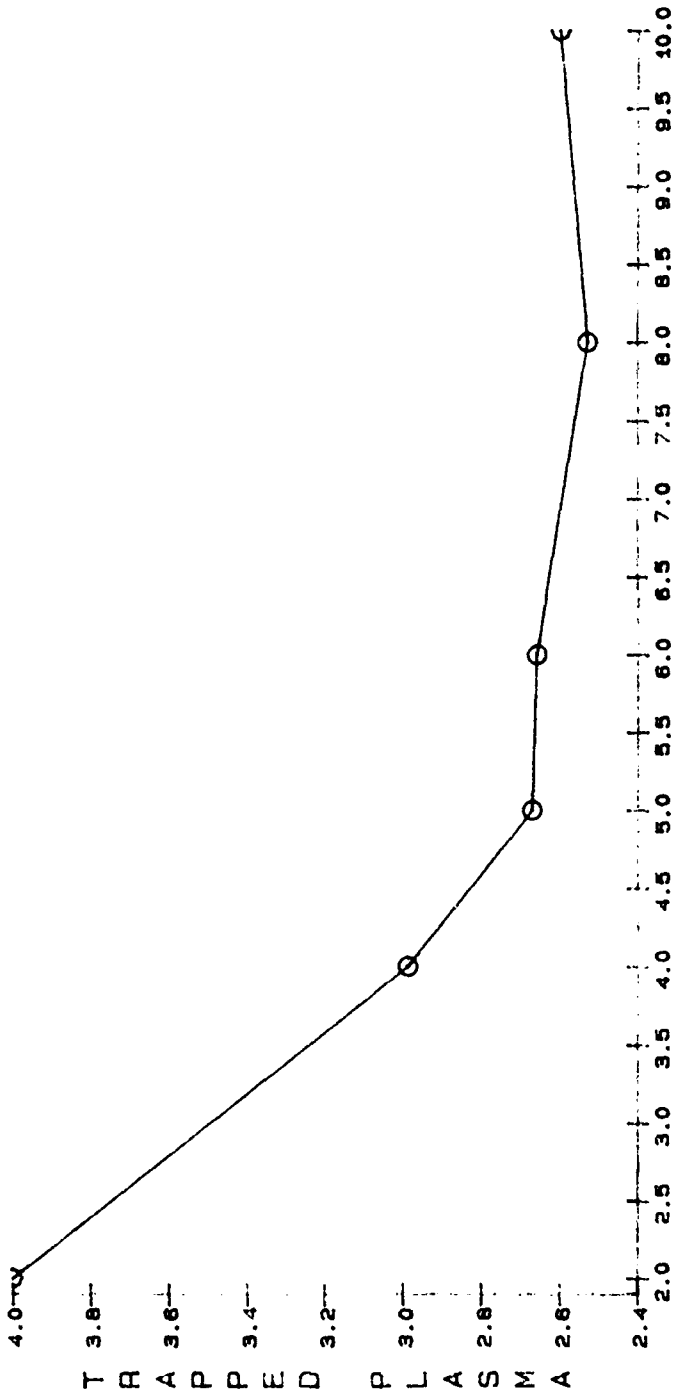


Figure 2. Optimum Packing Time (Relative Trapped Plasma Value)

separated into the plasma segment. However, this one millimeter loss of packed cells accounts for less than 4% of the total packed cell volume. A 65 mm column of whole blood with a hematocrit of 40% centrifuges to yield a 26 mm packed cell volume. One millimeter lost of the original 26 mm is exactly 3.8%. Consequently, trapped plasma values reported in this thesis are slightly lower than the true value. This error is small in comparison to the error produced when plasma, with its relatively high radioactivity, is lost or included in the packed cell segment.

^{131}I Loss From The Capillary Tube Breaking Process

Another concern is whether there is significant loss of plasma during the capillary tube breaking process. To evaluate this, ten replicate capillary tubes were prepared from one sample of healthy donor blood. For this study only, the capillary tubes were filled to less than 40 mm to preclude the loss of radiation counts above the crystal (See below Counting Problems). Whole uncentrifuged capillary tubes were counted. Each was then broken according to the usual protocol and the individual segments were counted again. The whole tube counts were compared to the added counts of the packed cell volume and

plasma segments (See Table 1). A paired t-test performed on the differences was not significant.

Gamma Counter

The packed cell and plasma segments were counted on a Packard Multi Prias 2 gamma counter (Packard Instrument Co., Downers Grove, IL 60515). Each segment was placed into a separate plastic gamma counter tube and capped. These tubes were then inserted into twelve-sample cassettes and loaded onto an input tray with an electromechanical sample changer. This electomechanical sample changer advances the cassettes toward two detectors one position at a time. When the cassette is properly aligned, an elevator mechanism simultaneously raises samples into corresponding detectors at the one and six sample position.

Gamma rays emitted from the sample interact with electrons in a sodium iodide crystal, which is part of the detector, producing scintillations of light. Since all of the gamma ray energy is deposited in the crystal, the amount of light produced is proportional to the amount of energy lost by the gamma ray. An important feature of the Multi Prias system and most other gamma counting systems is that it not only counts the gamma rays, but sorts them according to their energies. This leads to a peak in the

TABLE 1:

RADIATION LOSS FROM CAPILLARY TUBE BREAKING PROCESS

	TOTAL TUBE COUNTS	ADDED SEGMENTS COUNTS	DIFFERENCE
	50805	51001	-196
	53343	54634	-1291
	51498	51092	+406
	49628	49774	-146
	52806	53114	-308
	45114	44652	+462
	37855	37092	+759
	45026	45336	-310
	50083	50361	-278
	50428	50471	-47
Ave.	48659	48753	-95 (0.2%)

PAIRED T TEST:

SIGNIFICANCE LEVEL = .6074
T = -.5304

final signal spectrum, called the photopeak, which is characteristic of each radiation source. The photopeak for the most important gamma emission on disintegration of ^{131}I is 364 keV (14). Therefore, the upper threshold of the gamma counter was set at 450 keV and the lower at 250 keV to capture all gamma rays of interest.

Each sample is counted simultaneously for a pre-set time by windows A and B of the same crystal. These two counts are then averaged. When counting is terminated, the sample tubes are lowered back into the cassette and the cassette is advanced to the next position.

Counting Time

The total number of radioactive events counted is based on the precision desired. In this project, a maximum of 3% sample error rate was chosen. For convenience, a fixed amount of ^{131}I was added to every specimen. This left the counting time as a floating variable. Convenient nomographs exist which allow one to calculate quickly the optimum counting time in order to achieve the precision required (See Figure 3). The fixed addition of ^{131}I HSA (See below ^{131}I Human Serum Albumin) resulted in packed cell volume counts just above 1,000 cpm. Background counts measured prior to each assay were approximately 200 cpm. The optimum counting time based on

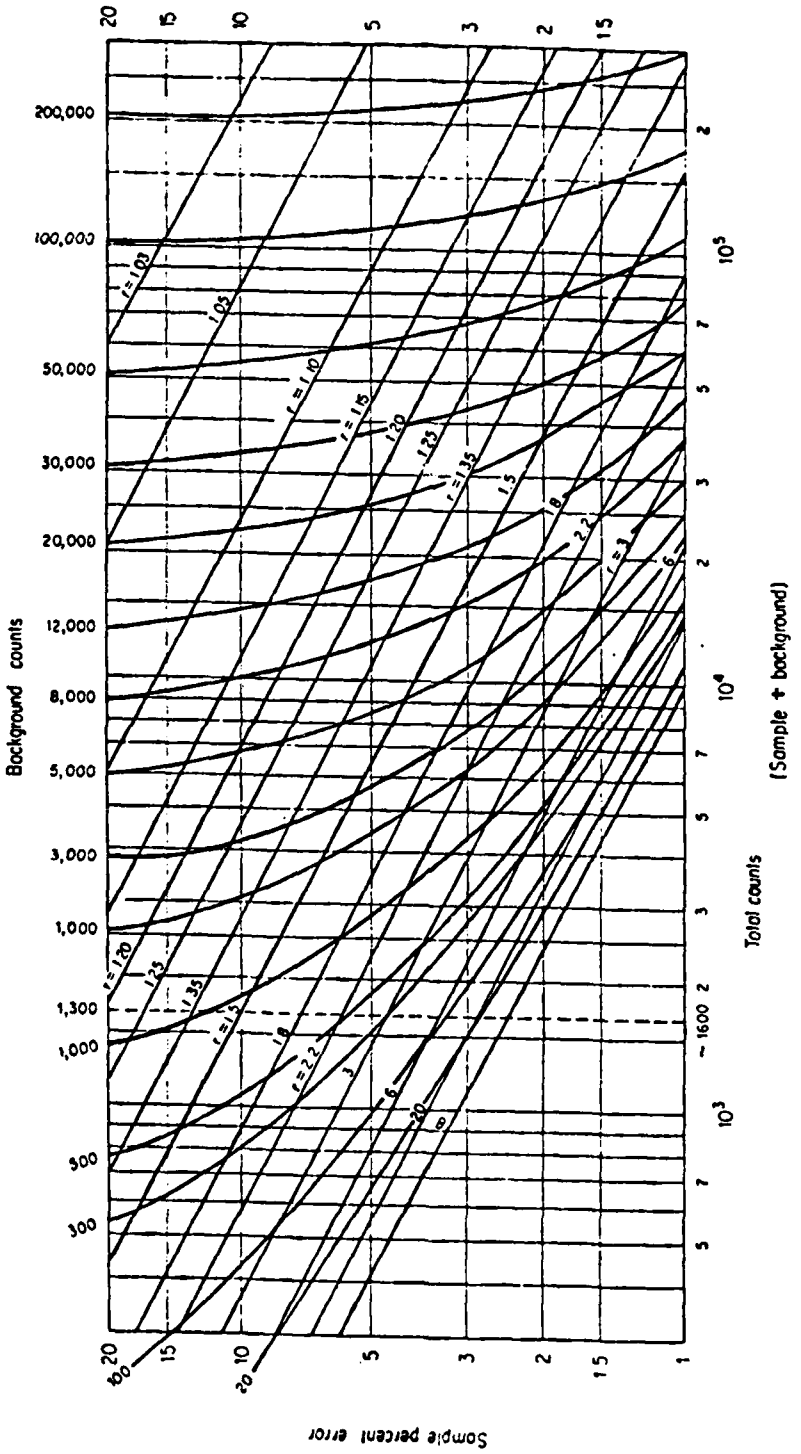


Figure 3. Optimal Counts Chart

these variables is estimated from the proper r diagonal line:

r = (sample cpm + background cpm)/background cpm

In this case r = (1000 + 200)/200 = 6. In order to achieve a precision of ±3%, we must first find the intersection of the horizontal r = 6 line with the chosen sample percent error. Tracing directly down from this intersection, we see that the total number of counts (sample + background) needed is 2,250. Since we already know the sample and background are emitting 1,200 cpm, we determine that 1.88 minutes are the minimum time needed (2,250/1,200 = 1.88). We can estimate the optimum background counting time by interpolating the total background counts that correspond with the original intersection. In this case, the total background counts needed is about 200. Since we know that the background rate is 200 cpm, only one minute of background counting time is needed for 3% precision. For convenience, the background and sample counting times were routinely set at two minutes. Whenever any segment count fell below 1,000 cpm, an appropriate sample counting time was determined from the Optimal Counts Chart.

Counting Problems

Two problems required further contraints on the counting methodology.

Geometry

The first geometry problem was crystal length. The sodium iodide crystal is cube shaped with an empty central core where the specimen is inserted. The total crystal length is approximately two inches. However, the bottom of the specimen tube is inserted one inch up into the crystal. This leaves about one inch of effective crystal counting length. Specimens approaching this height or more will show erroneously low counts since many of the gamma rays will exit at an angle above the crystal and not be counted.

To assess the importance of this geometry problem in my studies, I placed glass capillary segments with either packed cells or plasma "in toto" into the gamma counter tubes. Long lengths of plasma did not prove to be a problem because the plasma would then drain down out of the capillary tube into the base of the gamma counting tube. But the packed cell lengths had to be broken into smaller segments whenever the length was greater than 44

mm. This maximum height was determined by measuring the counts per minute of various lengths of plasma (See Table 2). In this particular study, plasma only was drawn into capillary tubes and one end sealed. These lengths were then counted in the gamma counter. Each plasma length was plotted against its resultant count. A linear correlation is exhibited up to the 44 mm length. Above this length less-than-expected counts were measured. When the plasma lengths were broken into smaller segments and recounted, higher counts which are linear were measured. The missing counts are those gamma rays exiting above the crystal.

Background Variation

The second problem encountered was a fluctuating background count. The gamma counter detects some background radiation even though the counting crystal is shielded. Background radiation emanates from the earth's crust and from cosmic radiation (16). The remaining background is from those specimens on the input/output tray not presently being counted. With all specimens far removed from the gamma counter, the background averaged about 50 cpm. On the other hand, when the input/output tray was fully loaded with radiolabeled specimens the background would rise to as high as 500 cpm. Although this background was measured prior to each assay and

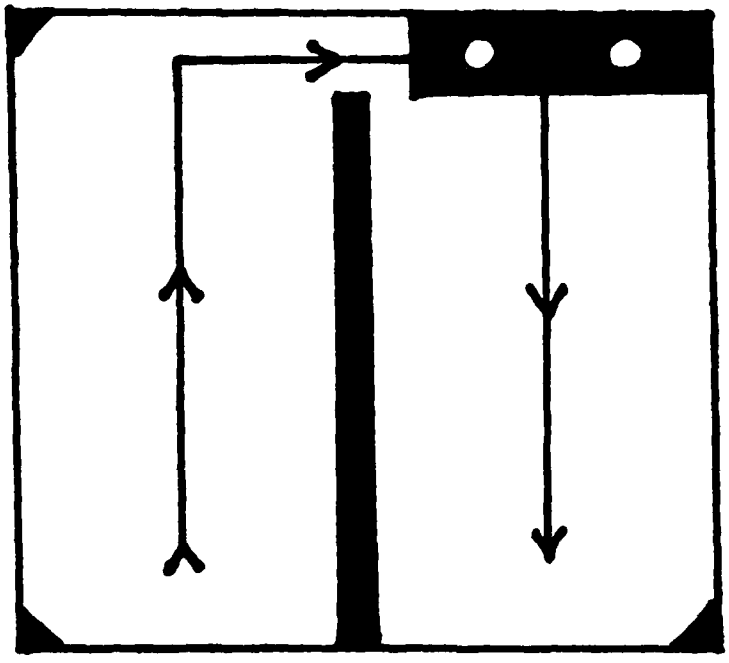
TABLE 2:
RADIATION LOSS FROM EXCESSIVE SEGMENT LENGTHS

LENGTH (mm)	CPM	CPM/LENGTH	CPM	CPM/LENGTH
	WHOLE TUBES		BROKEN TUBES	
1. 17.8	26476	1487		
2. 22.0	31836	1447		
3. 24.8	36183	1459		
4. 27.8	40100	1442		
5. 31.1	45190	1453		
6. 36.9	51173	1387		
7. 38.8	54674	1409		
8. 41.6	57966	1393		
9. 42.5	59667	1404		
10. 44.0	60336	1371	62960	1431
11. 47.2	64035	1357	67968	1440
12. 52.8	68536	1298	74488	1410

subtracted from subsequent samples, the background would fluctuate as much as 50 cpm depending on the geometric arrangement of the specimens not being counted (See Figure 4). Whenever plasma segments were in very close proximity to the crystal, the background would rise. This fluctuation is due to the principle called the Inverse Square Law. Basically, this law states that the amount of radiation falls as a square of the distance from the detector (16).

Several attempts to increase crystal shielding with thin layers of lead foil did not appreciably decrease background counts. The fluctuation was finally minimized by narrowing the keV window of measurement from 250-450 keV to 325-400 keV. The total background count was lowered to 50 cpm and the fluctuation virtually disappeared. Sample counts were also decreased, but only by a small proportion. Why the decrease in sample counts was not proportional to the decrease in background is unclear since the count fluctuations were presumably related to variations in detecting ^{131}I. Fortunately, the drop in sample and background counts did not change the optimal counting time as estimated from the Optimal Counts Chart.

As an added precaution, blank cassettes were inserted between each cassette loaded with specimens. These blank cassettes served to keep specimens not being



MULTI PRIAS 2 INPUT/OUTPUT TRAY

Figure 4

counted further from the detectors. Finally, only two specimens were loaded per specimen rack, at the one and six sample position. In this configuration they were counted simultaneously and crosstalk was prevented by the double shielding effect of the two individually shielded crystals.

^{131}I Human Serum Albumin

The ^{131}I Human Serum Albumin (International Chemical and Nuclear Biomedicals, Inc., Irvine, CA 92715) was supplied with a specific activity of 10 uCi/mg HSA. The human serum albumin had a concentration of 100 uCi/mL and 10 mg HSA/mL. Prior to use it was thawed and well mixed. Throughout the remainder of the week, it was stored in a refrigerator at 4^{o}C.

Two drops of ^{131}I HSA were added per mL whole blood to each sample. The drops were delivered by a 23 gauge by 3/4" Butterfly Infusion Set (Abbott Hospitals Inc., North Chicago, IL 60064) attached to a 1 cc tuberculin syringe (Becton, Dickinson, and Co., Rutherford, NJ 07070) held vertically above the sample. The samples were then mixed for a minimum of three minutes, after which capillary tubes were filled as previously described.

Using less than two drops of ^{131}I HSA would have required much longer counting times to maintain the chosen

sample error rate. Conversely, if more than two drops were added the plasma segment count rate sometimes exceeded 100,000 cpm. Counts above 100,000 cpm exceed the coincidence resolution of the Multi Prias 2 gamma counter (14).

Formula

Net counts from the gamma counter and measured microhematocrit values were used to derive a relative trapped plasma value. This value is "relative" because it is dependent on the hematocrit level. Correctly stated, the relative trapped plasma is the percentage of trapped plasma per unit of packed cell volume (6). The formula is:

$$\text{Relative trapped plasma} = \frac{(\text{pcv cpm})(1-\text{hct})(100)}{(\text{plasma cpm})(\text{hct})}$$

This relative trapped plasma formula is derived from the total trapped plasma formula. The total trapped plasma is the trapped plasma expressed as a percentage of the entire capillary tube. This formula is:

$$\text{tp} = \frac{(\text{pcv cpm})}{(\text{pcv cpm} + \text{plasma cpm})} \times [\text{tp} + \text{plasmacrit}]$$

$\wedge$ $\wedge$

% of pcv counts total plasma volume

plasmacrit = (1-hct)

This formula is algebraically manipulated as follows:

$$tp(\text{pcv cpm} + \text{plasma cpm}) = (\text{pcv cpm})[(tp + (1-hct)]$$

$$(tp)(\text{pcv cpm})+(tp)(\text{plasma cpm})=(\text{pcv cpm})(tp)+(\text{pcv cpm})(1-hct)$$

$$(tp)(\text{plasma cpm}) = (\text{pcv cpm})(1-hct)$$

$$tp = \frac{(\text{pcv cpm})(1-hct)}{(\text{plasma cpm})}$$

However, it is difficult to compare total trapped plasma values between different samples because the value is dependent on the hematocrit of each sample. For example, a hematocrit of 40% contains more total trapped plasma than a hematocrit of 20%. In order to allow sample to sample comparison, the total trapped plasma value is divided by the hematocrit level and is expressed as a percentage. This results in the relative trapped plasma value expressed as a percentage of the packed cell volume.

$$\text{Relative tp} = \frac{tp \times 100}{hct} = \frac{(\text{pcv cpm})(1-hct)(100)}{(hct)(\text{plasma cpm})}$$

Sephadex Bead Filtration

One potiential problem relating to the ^{131}I method for determination of trapped plasma is that the

^{131}I HSA might be supplied with a portion of free ^{131}I. In such a case, the free ^{131}I could possibly bind to the red cell surface or enter the cell and equilibrate with intracellular water. The result would increase the packed cell column counts artificially, netting an erroneously high relative trapped plasma value. To prove that all supplied ^{131}I was bound to the albumin, two experiments were carried out. The first was a Sephadex bead filtration. G-50 Sephadex beads (Pharmacia Fine Chemicals, Piscataway, NJ 08854) were reconstituted in a 0.1 mol/L phosphate buffer solution (NaH_2PO4/Na_2HPO4) overnight. The beads were then degassed for twenty minutes and carefully poured into a 10 mL glass serologic pipet with glass wool in the tip. Sufficient beads were added to create a column of about 1 1/2 centimeters. A small rubber hose and clamp were attached to the pipet tip to control the flow. A 1 mL aliquot of ^{131}I HSA mixed with the phosphate buffer solution was added to the top of the bead column. This aliquot contained approximately 150,000 cpm. ^{131}I HSA that had been thawed four days was used as this was the maximum age of any ^{131}I HSA in any phase of this project. Three drop aliquots were delivered into separate gamma counter tubes and counted on the Multi Prias 2 gamma counter. Sufficient phosphate buffer solution was added to the top of the bead column to keep the topmost beads continuously immersed. The aliquot

number was plotted against its corresponding count and the result is a graph with a single peak (See Figure 5). A single peak demonstrates a single population, namely the ^{131}I HSA in bound form. Free ^{131}I is a smaller molecule than ^{131}I HSA. Had there been any free ^{131}I, it would have entered the Sephadex beads and been retarded in its flow through the column. This second population of free ^{131}I would then have been eluted at higher aliquot numbers culminating in a second peak.

A second experiment demonstrated that the ^{131}I HSA does not significantly bind to the red cell surfaces. One drop of fresh and one drop of four-day-old ^{131}I HSA was added to one milliliter samples of whole blood and mixed. After one hour of room temperature incubation the cells were washed four times with normal saline and decanted. Red cell buttons were then counted on the gamma counter. The remaining radiation was less than 0.2% of the original counts.

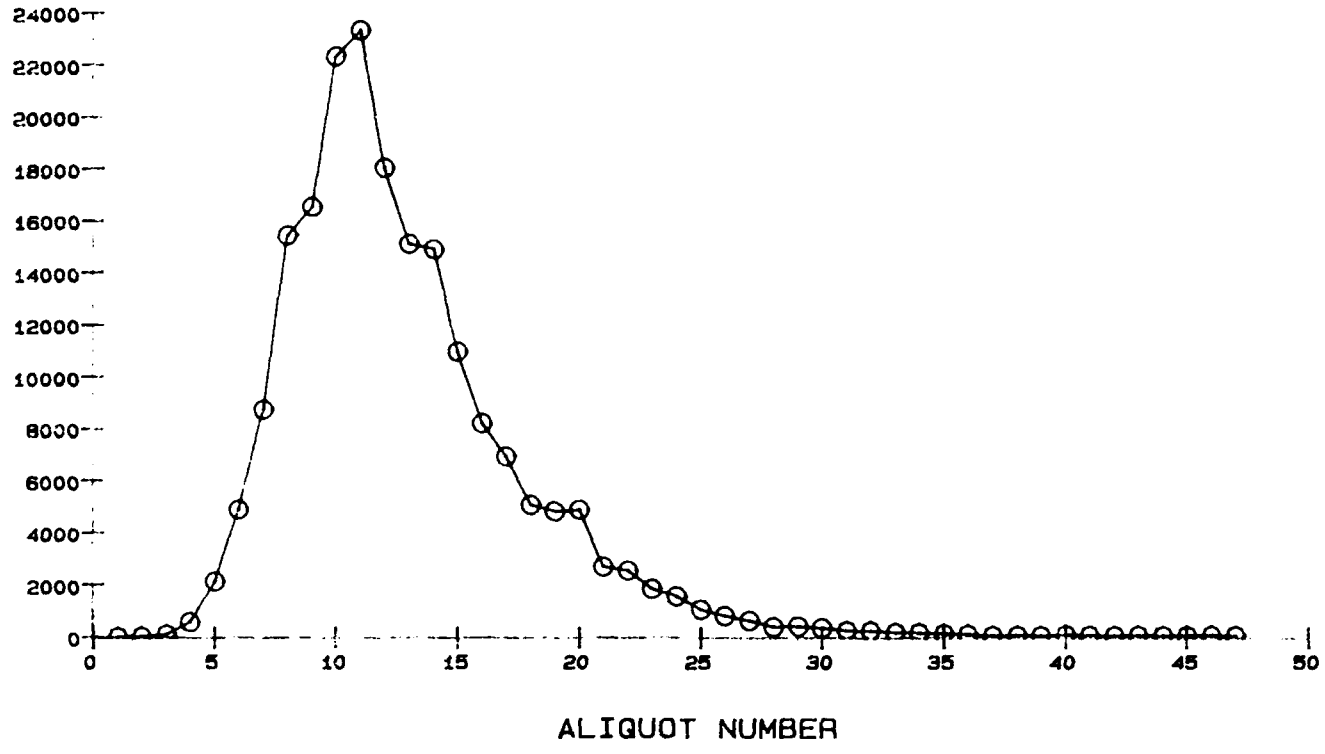


Figure 5. Sephadex Column Separation

CHAPTER THREE

RESULTS AND DISCUSSION

Relative Trapped Plasma Measurements

Standard Deviation of the Method

The standard deviation of the method was calculated from ten determinations of relative trapped plasma from a single healthy donor. The standard deviation of the method is $\pm$ 0.37%.

Standard Deviation of the Method (in %)

Number	Relative Trapped Plasma	Number	Relative Trapped Plasma
1.	3.5	6.	3.6
2.	3.6	7.	3.3
3.	3.4	8.	3.5
4.	3.3	9.	3.6
5.	3.7	10.	3.4
Ave. =	3.5	S.D. =	$\pm$0.37

Chaplin and Mollison (5) demonstrated that the top third of the packed cell column contains more trapped plasma than the remaining bottom two-thirds. The break of the capillary tube in my study is made at the topmost portion of the packed cell column, the area of greatest trapped plasma concentration. Thus, the variability in the relative trapped plasma value might be a result of the small deviations in the break point when each capillary tube is broken.

The Effect of Anticoagulant

The anticoagulant used in this project is K_3EDTA, the most commonly used anticoagulant for hematocrit determinations in the United States (cited in 17). The earlier cited investigators used heparin (6) and K_2EDTA in their studies (2,7). Lines and Grace in 1984 (17) reported that when K_3EDTA was used to anticoagulate blood samples, the centrifuged hemtocrits were 2% lower than when blood samples were anticoagulated with K_2EDTA or heparin. They concluded that the lower hematocrit values observed with K_3EDTA were caused by cell shrinkage due to the osmotic effect of the additional potassium cation. Another explanation for these findings might be that K_3EDTA in some way decreases the relative trapped plasma. We examined this possibility by drawing two samples each

from five healthy individuals, one each into K_3EDTA and K_2EDTA. Centrifuged hematocrits and relative trapped plasma values were determined (See Table 3). Our data corroborate the work of Lines and Grace. In this study the K_3EDTA anticoagulated samples result in a hematocrit 2.5% lower, on the average, than in samples anticoagulated with K_2EDTA. The amount of trapped plasma in samples anticoagulated with K_3EDTA is virtually identical to the amount in samples anticoagulated with K_2EDTA. Therefore, it appears that the hematocrit difference resulting from the use of these two anticoagulants relates to osmotic effects alone or to osmotic effects and factors other than changes in trapped plasma.

The Effect of Incubation

Two of the literature sources (2,8) report that relative trapped plasma values increase with increasing time from collection to analysis. To determine the effect delay in analysis has on the relative trapped plasma values, we analyzed specimens at varying times after collection.

For this study, two samples each were obtained from three healthy volunteers. In order to simulate the situation when clinical specimens are collected, one sample of each pair was incubated at room temperature and

TABLE 3:

ANTICOAGULANT EFFECT

	HEMATOCRIT		TRAPPED PLASMA	
	K_2EDTA	K_3EDTA	K_2EDTA	K_3EDTA
TA	45.5	44.6	2.2	2.6
DH	37.8	36.8	1.8	2.0
MW	42.3	41.3	2.0	2.3
SH	41.0	39.8	2.3	2.3
JB	40.0	39.0	2.2	2.4
AVE.	41.3	40.3	2.1	2.3

the other refrigerated at about 4°C. Prior to each assay, the refrigerated sample was warmed to about 21°C in a water bath. Relative trapped plasma values were determined at 30 minutes, 2 1/2 hour, 4 1/2 hour, 7 1/2 hour, and 24 hour time intervals. The effect of analysis delay is shown graphically in Figures 6 and 7. These graphs indicate that incubation increases relative trapped plasma values. Refrigerated specimens are affected less than unrefrigerated samples at each time interval. On the average, the relative trapped values from the room temperature samples increased on the average by 0.2% by the 7 1/2 hour interval and 0.5% after 24 hour incubation. The refrigerated samples increased only 0.2% in 7 1/2 hours and 0.3% in 24 hours.

The effect of incubation demonstrated by our data agrees with the studies by England, Walford, and Waters and Furth. Although England, et al demonstrated a more striking increase in relative trapped plasma with twenty-four hours incubation, their study also reported strikingly higher values for reference range and diseased blood. Furth reported only small increases of trapped plasma within the first twenty-four hours of incubation and then a larger increase in trapped plasma at 48 hours.

All specimens in this project, except one, were assayed within 7 1/2 hours. The lone exception is patient AN (See Table 7) whose sample was kept refrigerated for

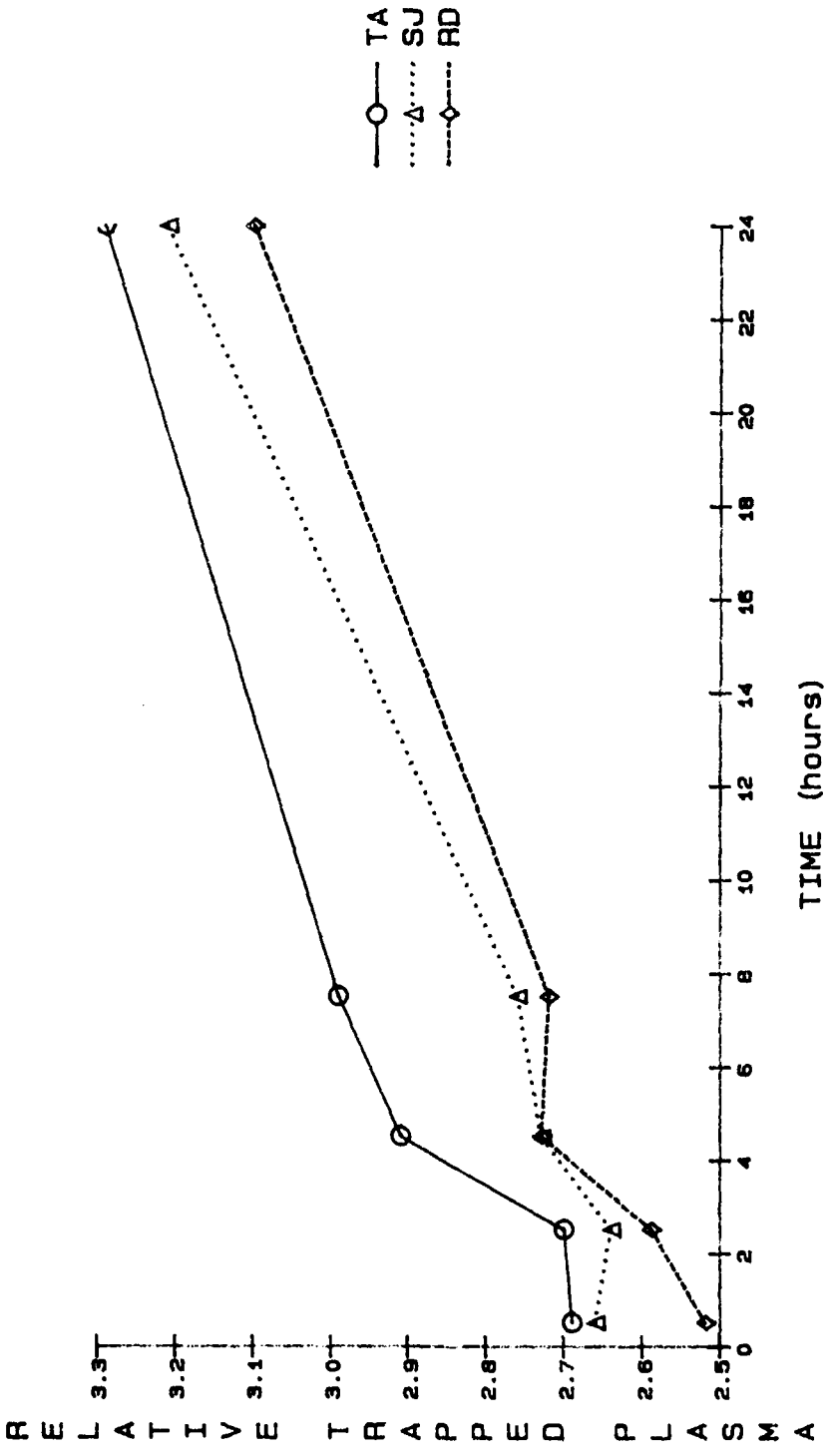


Figure 6. Effect of Incubation (Room Temperature)

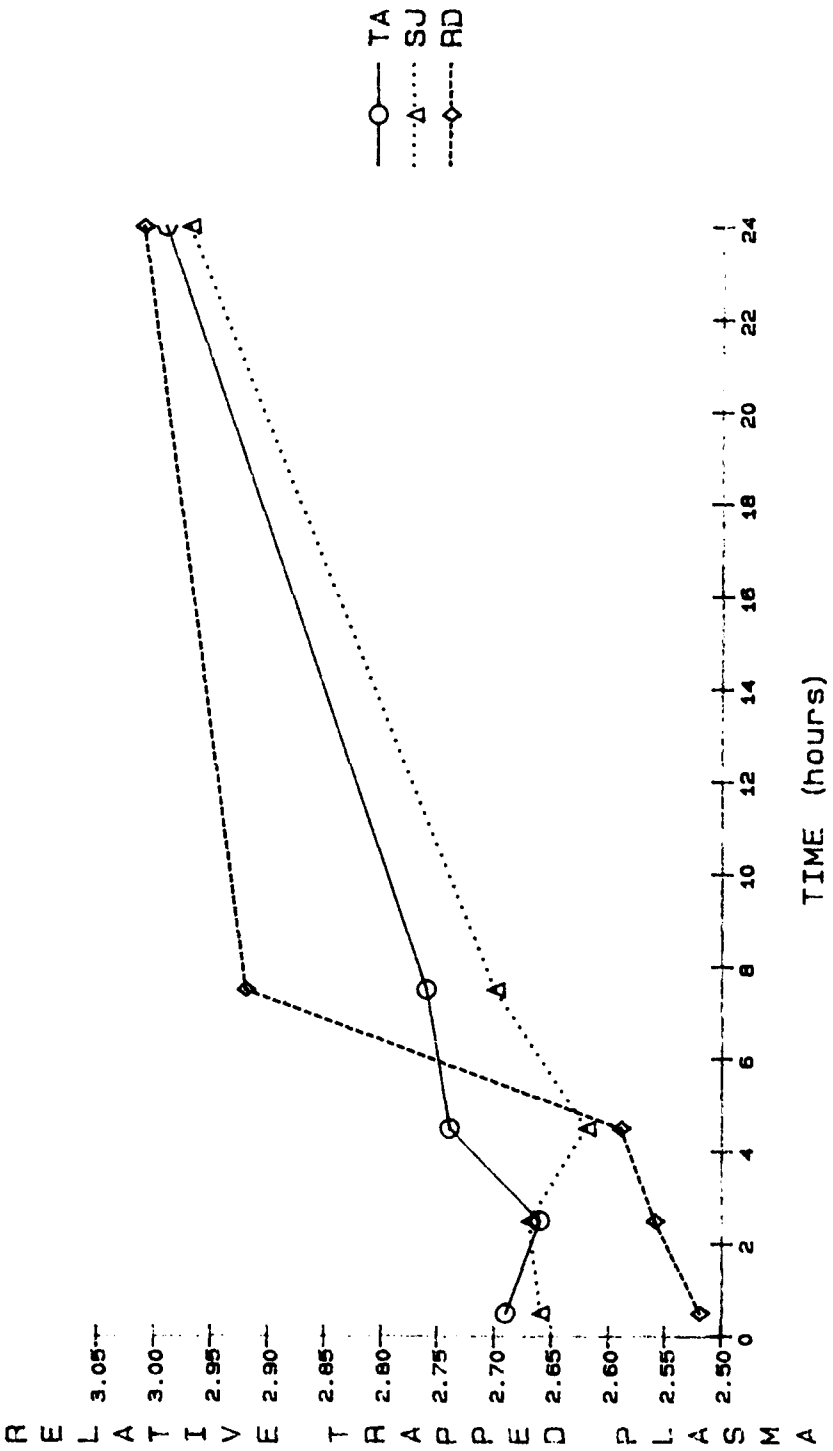


Figure 7. Effect of Incubation (Refrigeration)

about 18 hours. The remaining specimens were stored at room temperature. Thus, all values in this study are within 0.2% to 0.3% relative trapped plasma percent that would have been obtained if samples had been analyzed immediately.

The Effect of Hematocrit Level

All relative trapped plasma values listed in this thesis were derived from samples at their physiologic hematocrit levels. This physiologic hematocrit level was slightly diluted by the addition of radiolabeled albumin. Hematocrits in this study ranged from 36.1% to 44.2%.

The effect of hematocrit level on the amount of trapped plasma is disputed. Two groups of investigators (2,6) concluded there wasn't any effect. However, their conclusions could be challenged because they are based on limited hematocrit ranges and on samples from a limited number of subjects.

Chaplin and Mollison (5) demonstrated that higher hematocrits have higher relative trapped plamsa values. This is due to the "decrease in effective radius of centrifugation" at the top of the red cell column in the hematocrit centrifuge. Therefore, if each reference sample could be adjusted to the same hematocrit, (e.g. an average value for healthy subjects) any effect of

hematocrit level would be negated. While this manipulation is technically feasible, it would be an arduous task. Consequently, I have chosen to study the effect caused by varying hematocrit levels. Any effect of trapped plasma variability due to hematocrit level would be even more striking in samples from patients with red cell disorders. This is because of the wider range of hematocrits. The hematocrits of samples from patients with red cell disorders ranged from 19.3% to 56.3%.

To examine the effect of hematocrit level on the relative trapped plasma value, ten of the original reference range samples were further evaluated. Each of the specimens was divided into several aliquots. All aliquots were centrifuged for two minutes on an IEC Benchtop Centrifuge at the lowest speed setting, thus separating plasma from cells. Portions of plasma were subtracted from or added to the aliquots to create varying hematocrits from the same specimen. One aliquot was maintained at the original physiologic hematocrit level. The reconstituted aliquots were mixed again for three minutes. Hematocrit determinations and relative trapped plasma values were derived as previously described. The data for these experiments are shown graphically in Figures 8-17. In samples with hematocrit levels greater than 15%, there is a trend toward increasing relative trapped plasma with increasing hematocrit. This roughly

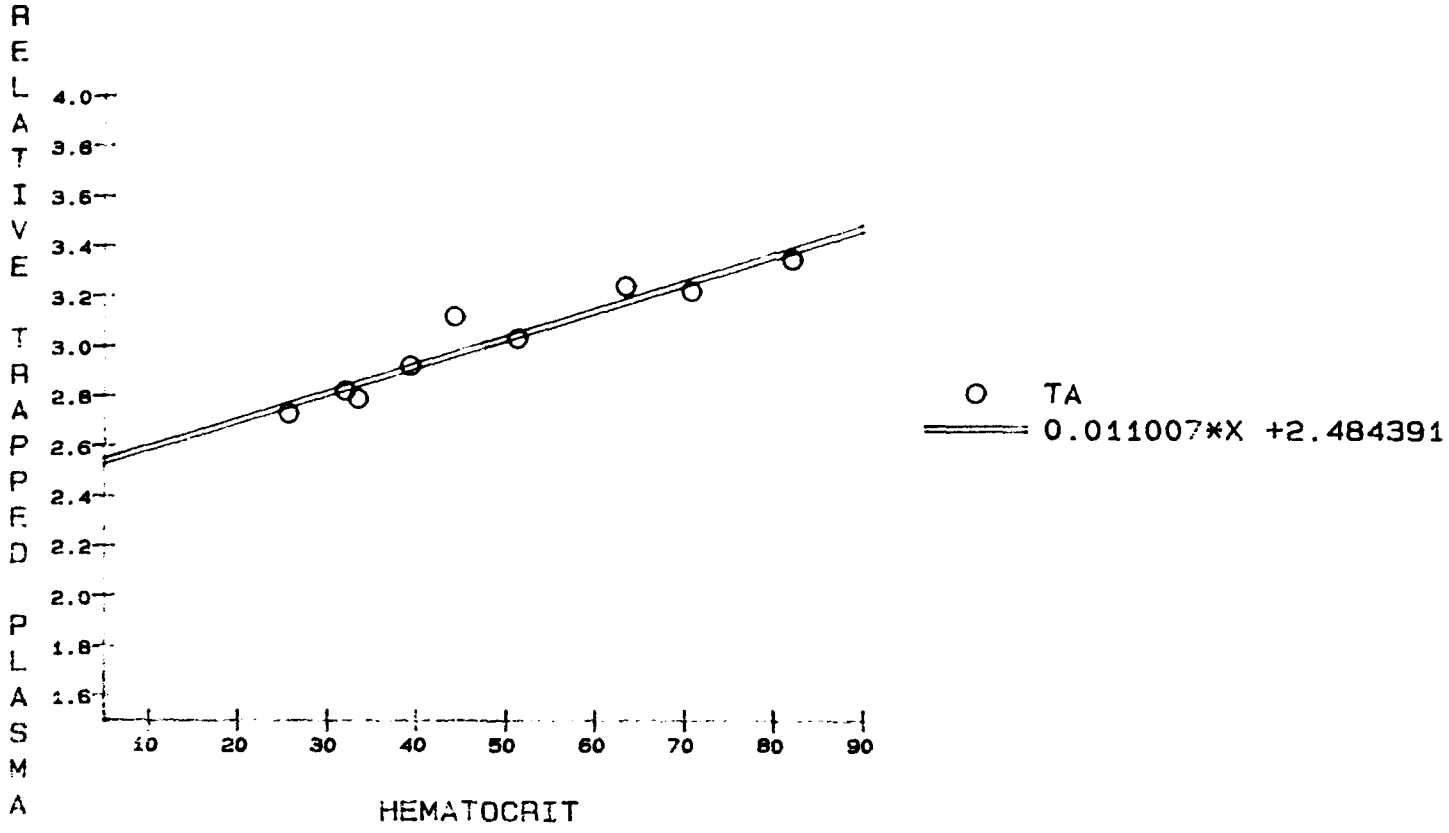


Figure 8. Hematocrit Level Effect (TA)

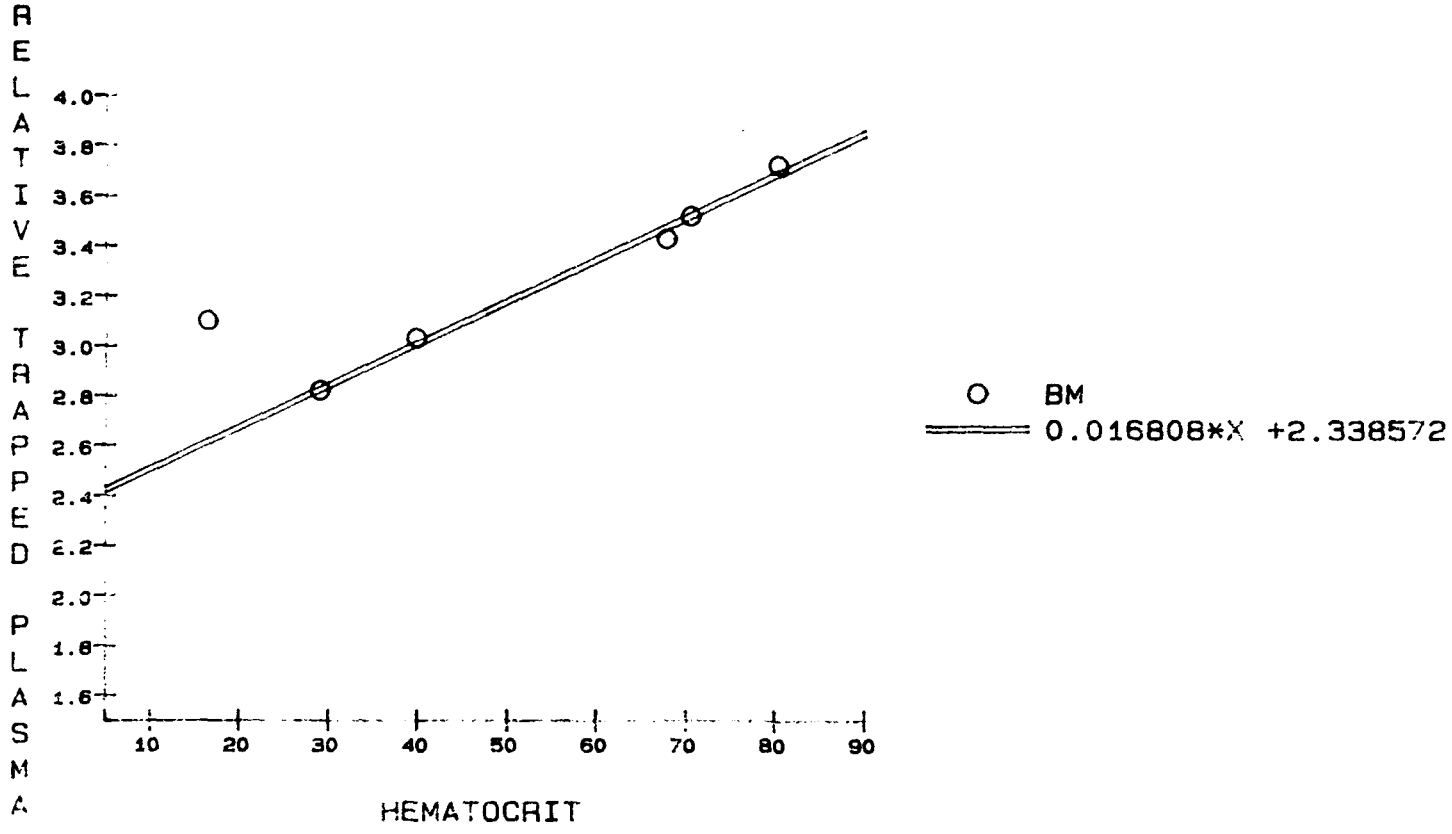


Figure 9. Hematocrit Level Effect (BM)

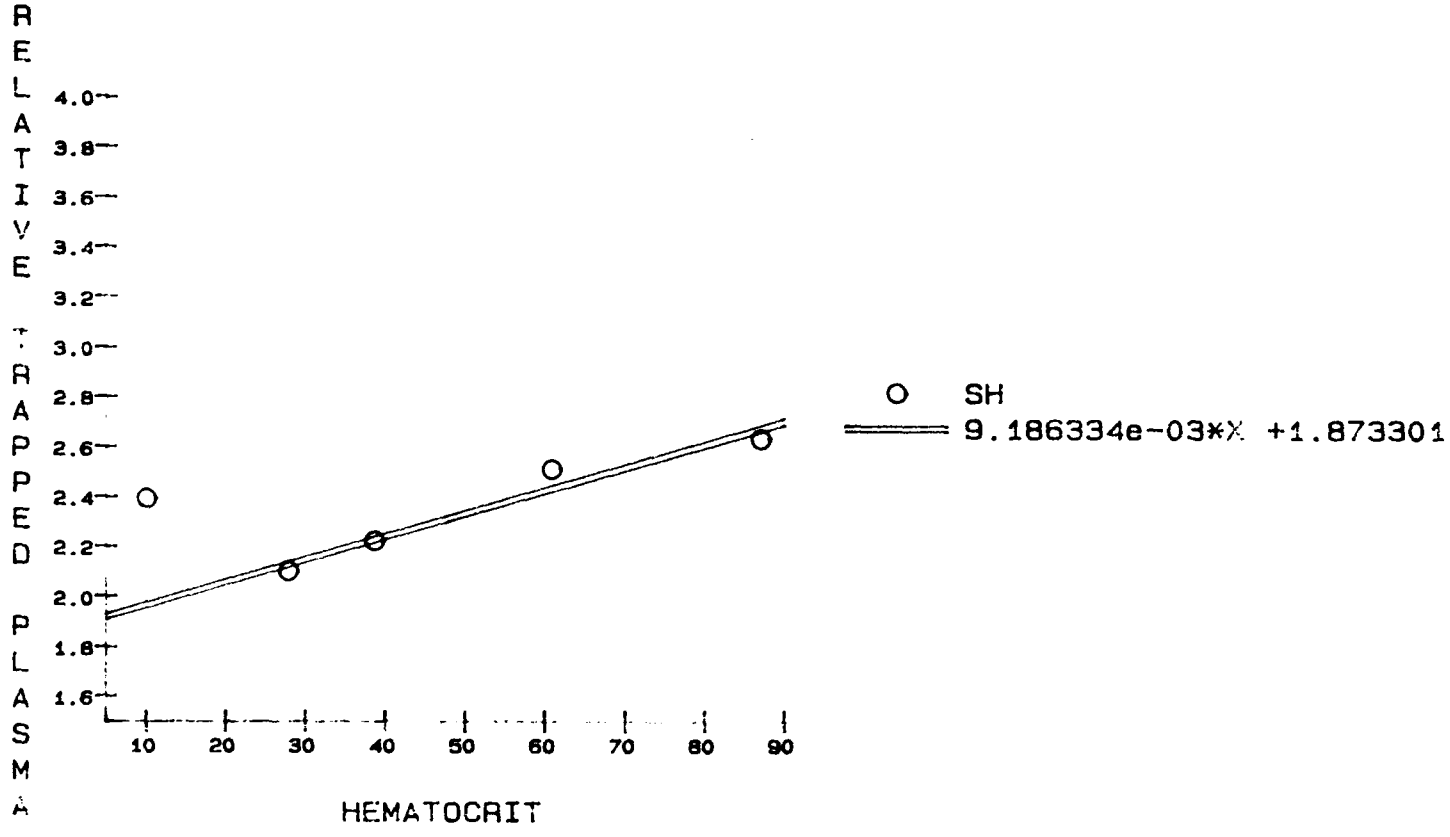


Figure 10. Hematocrit Level Effect (SH)

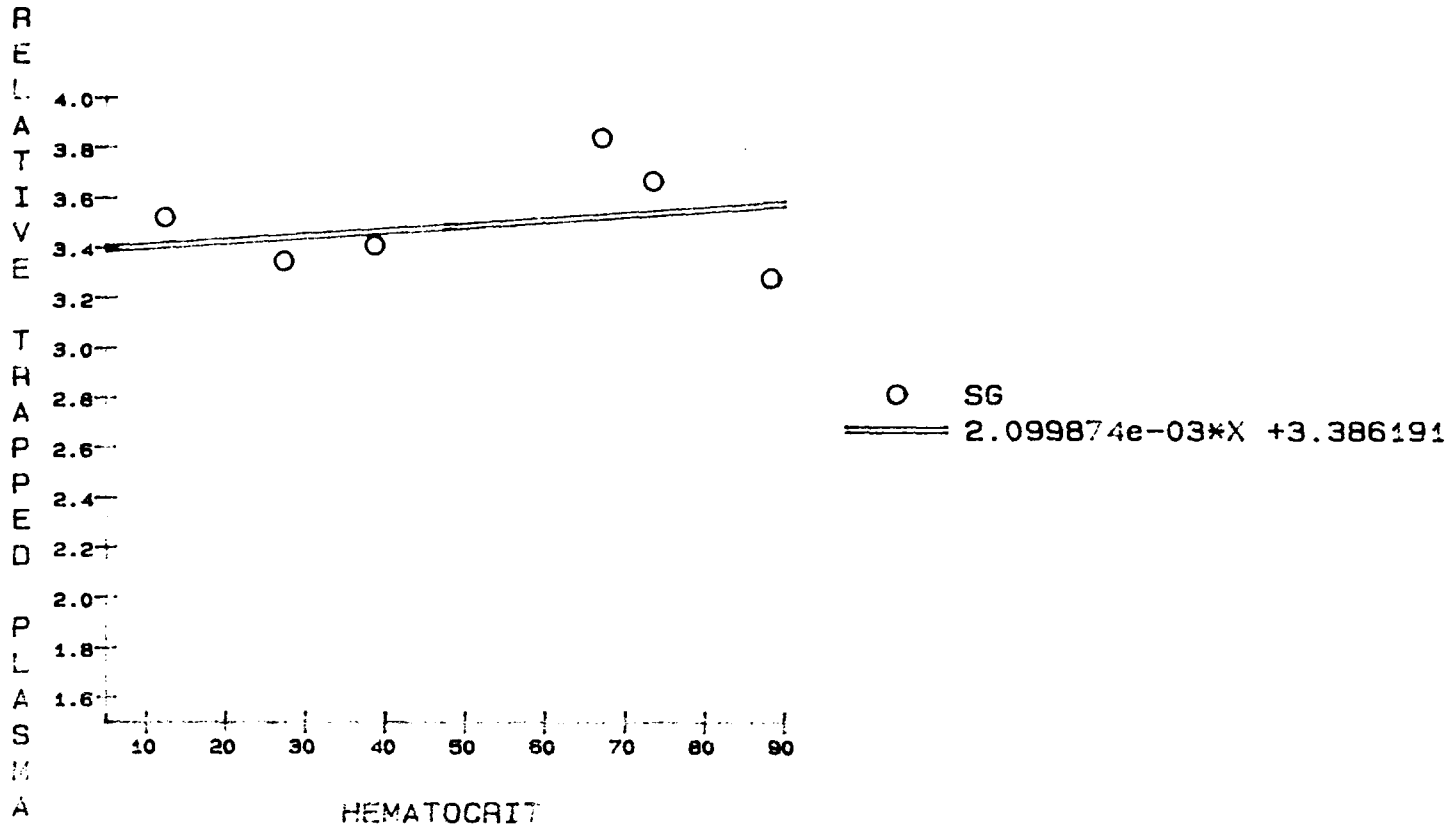


Figure 11. Hematocrit Level Effect (SG)

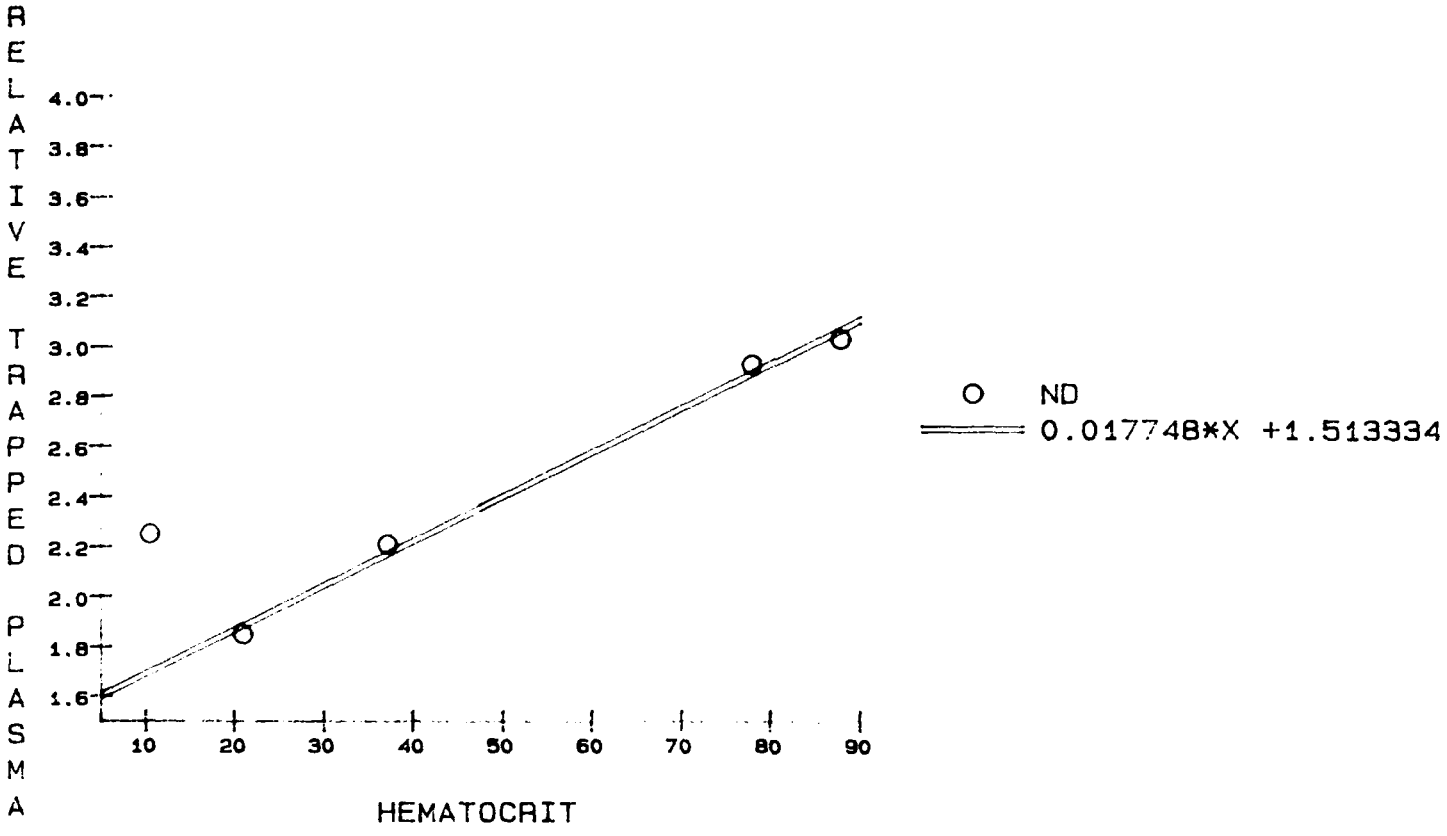


Figure 12. Hematocrit Level Effect (ND)

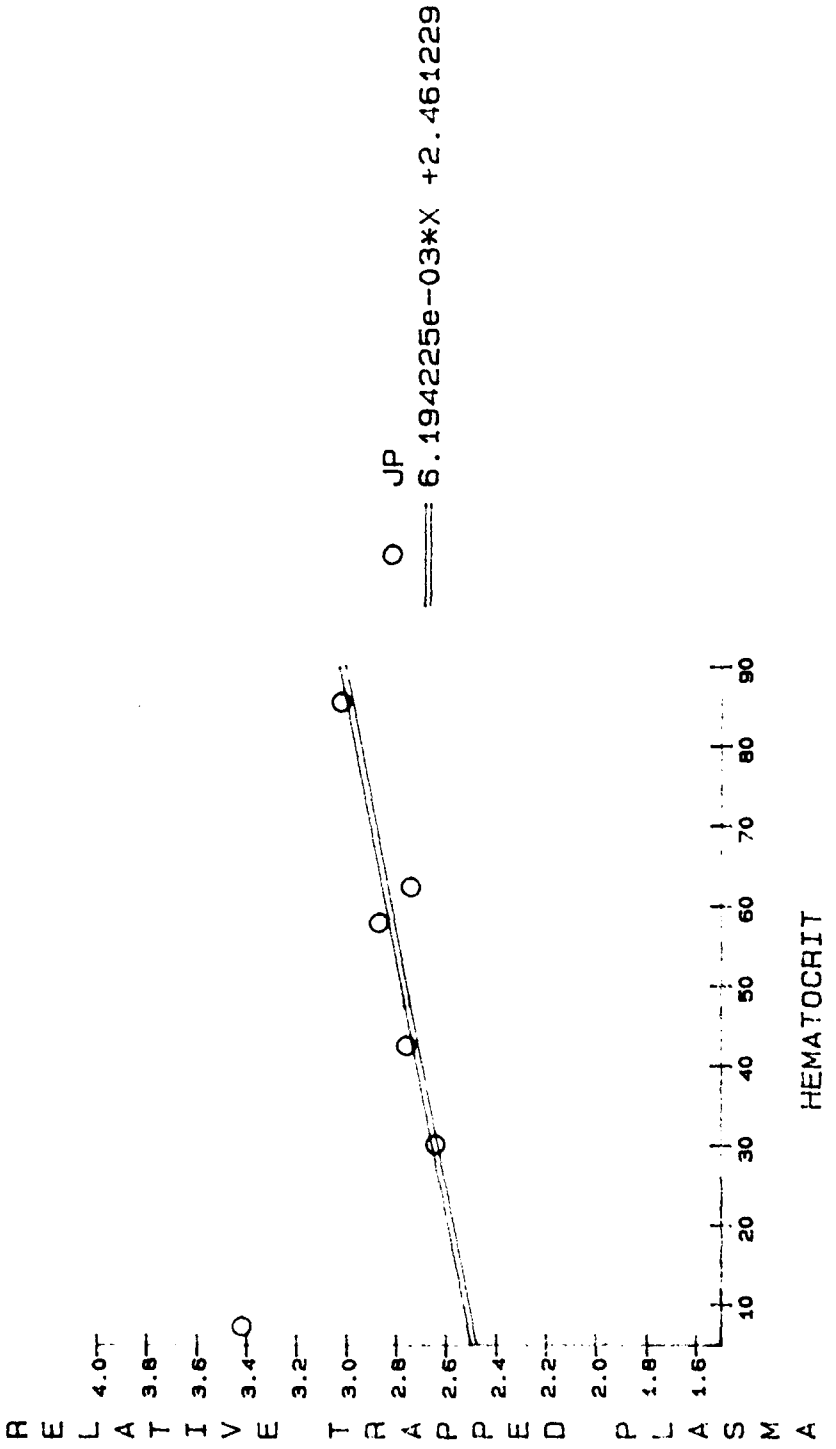


Figure 13. Hematocrit Level Effect (JP)

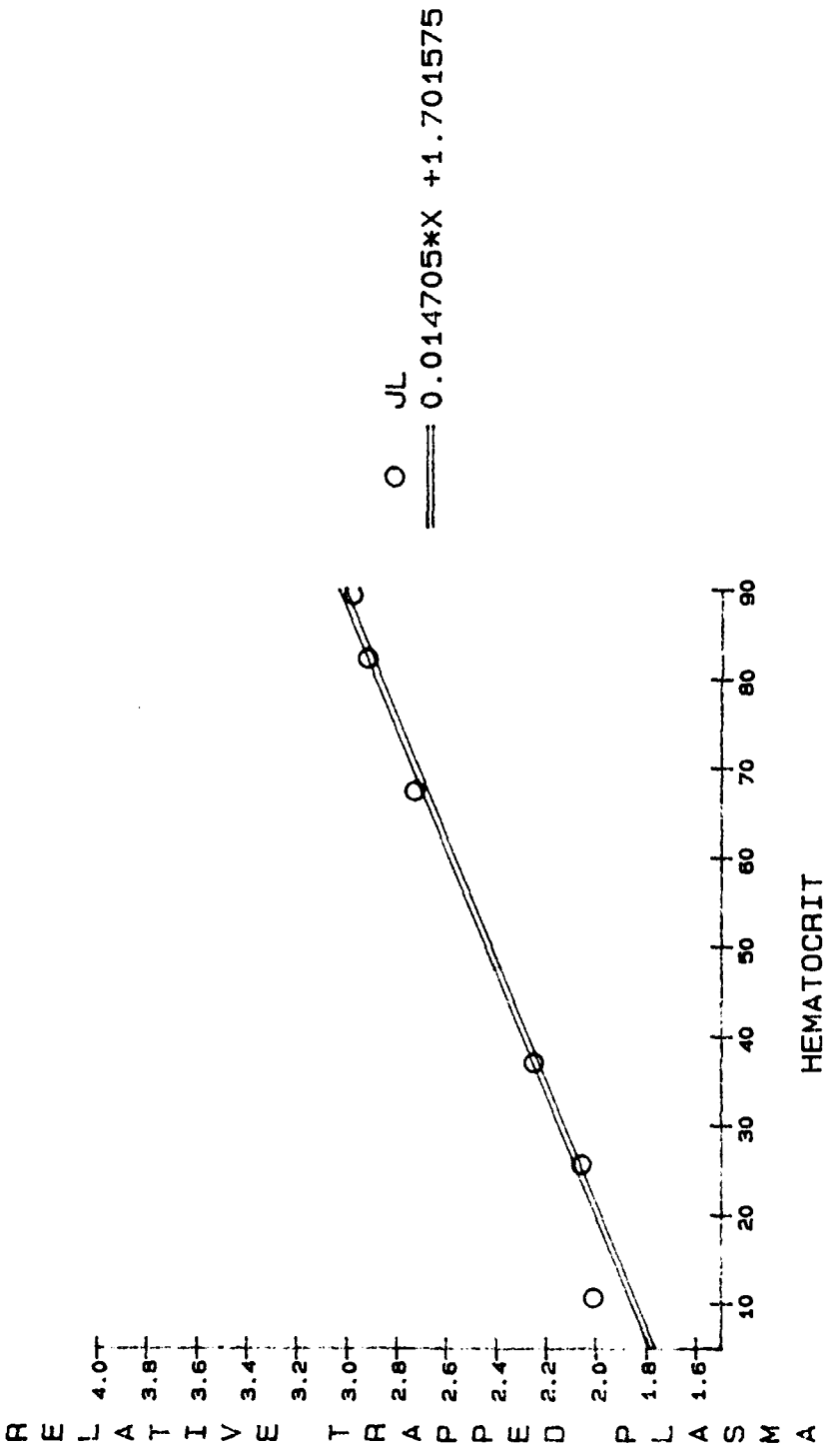


Figure 14. Hematocrit Level Effect (JL)

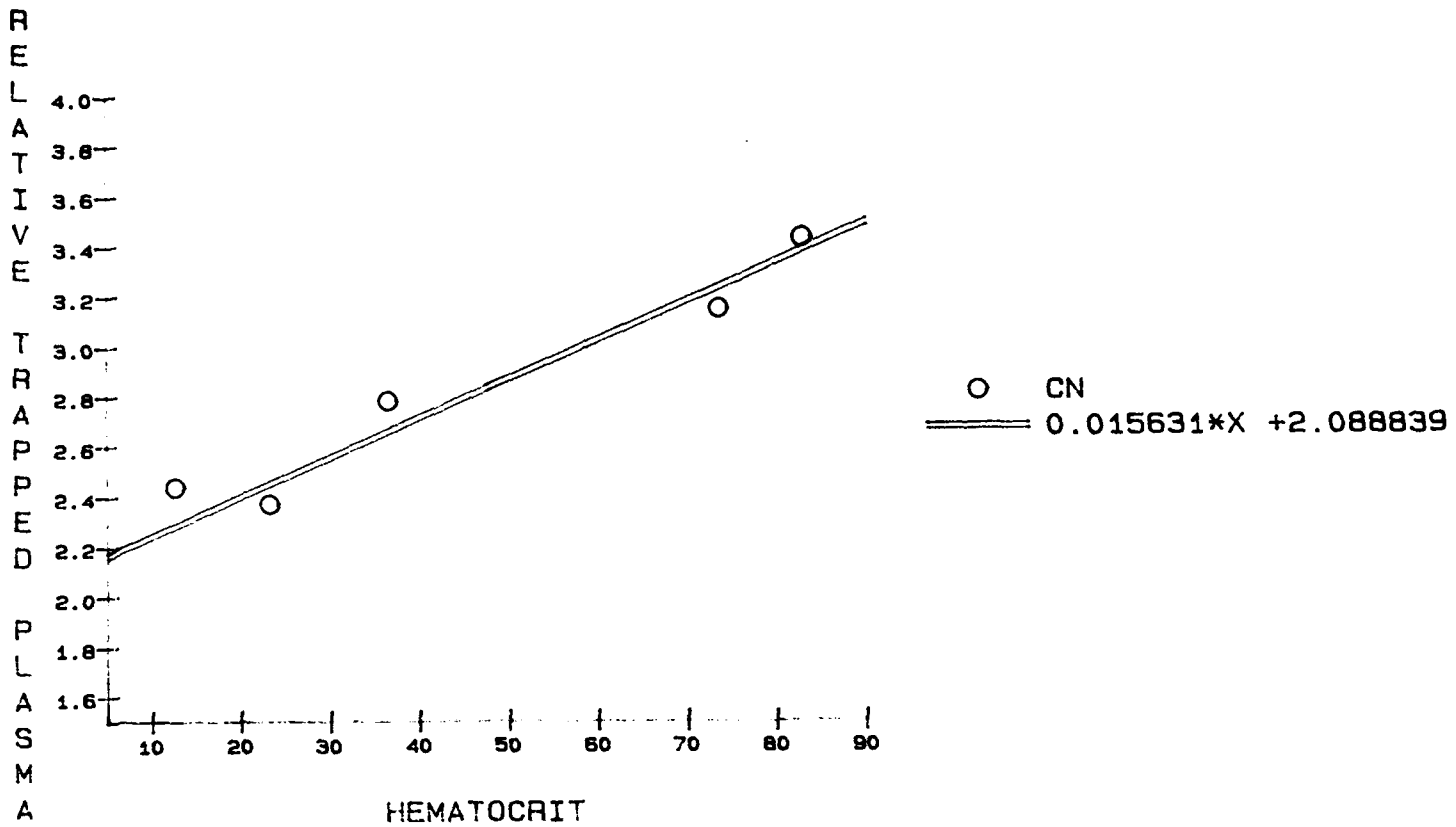


Figure 15. Hematocrit Level Effect (CN)

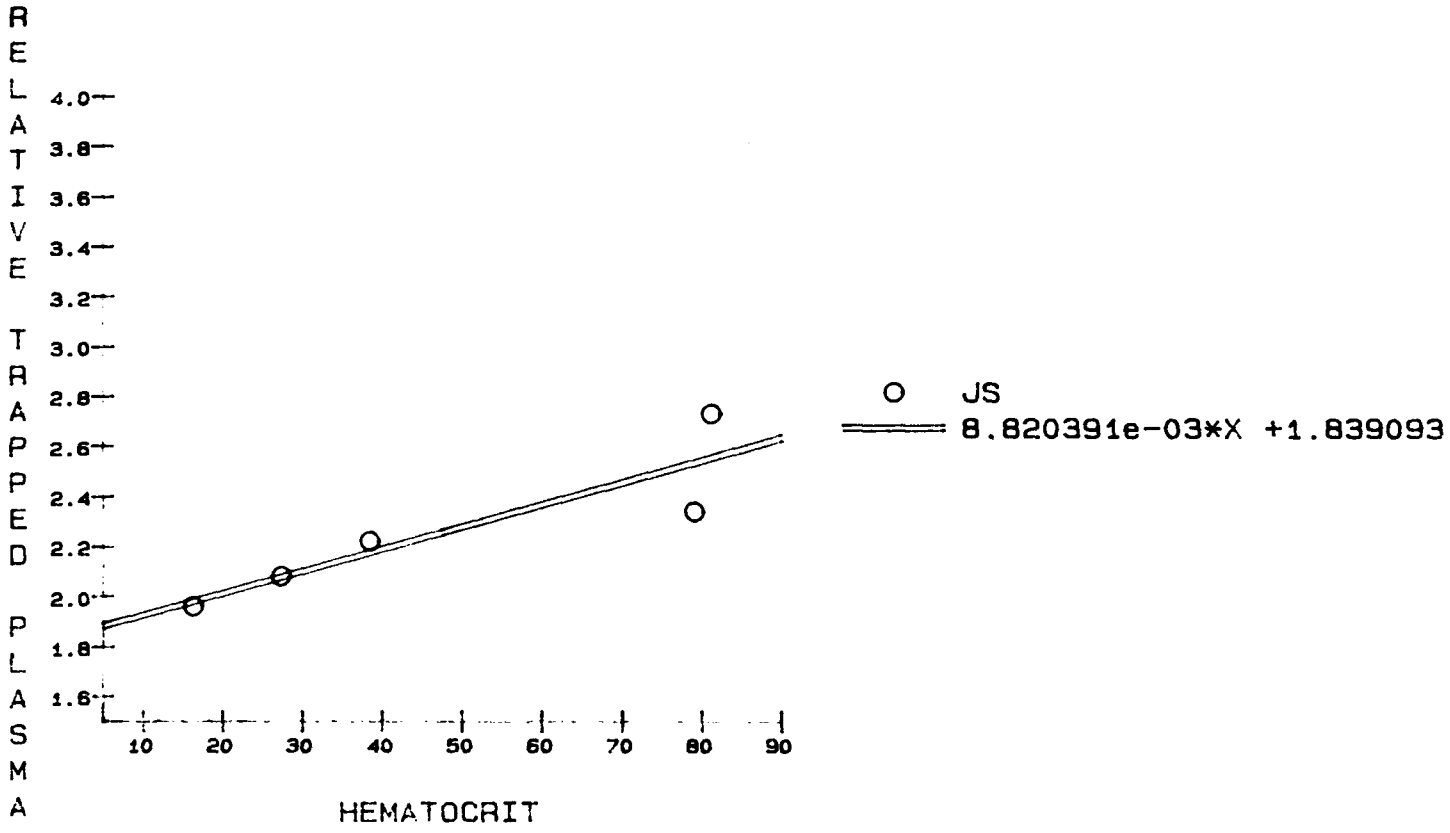


Figure 16. Hematocrit Level Effect (JS)

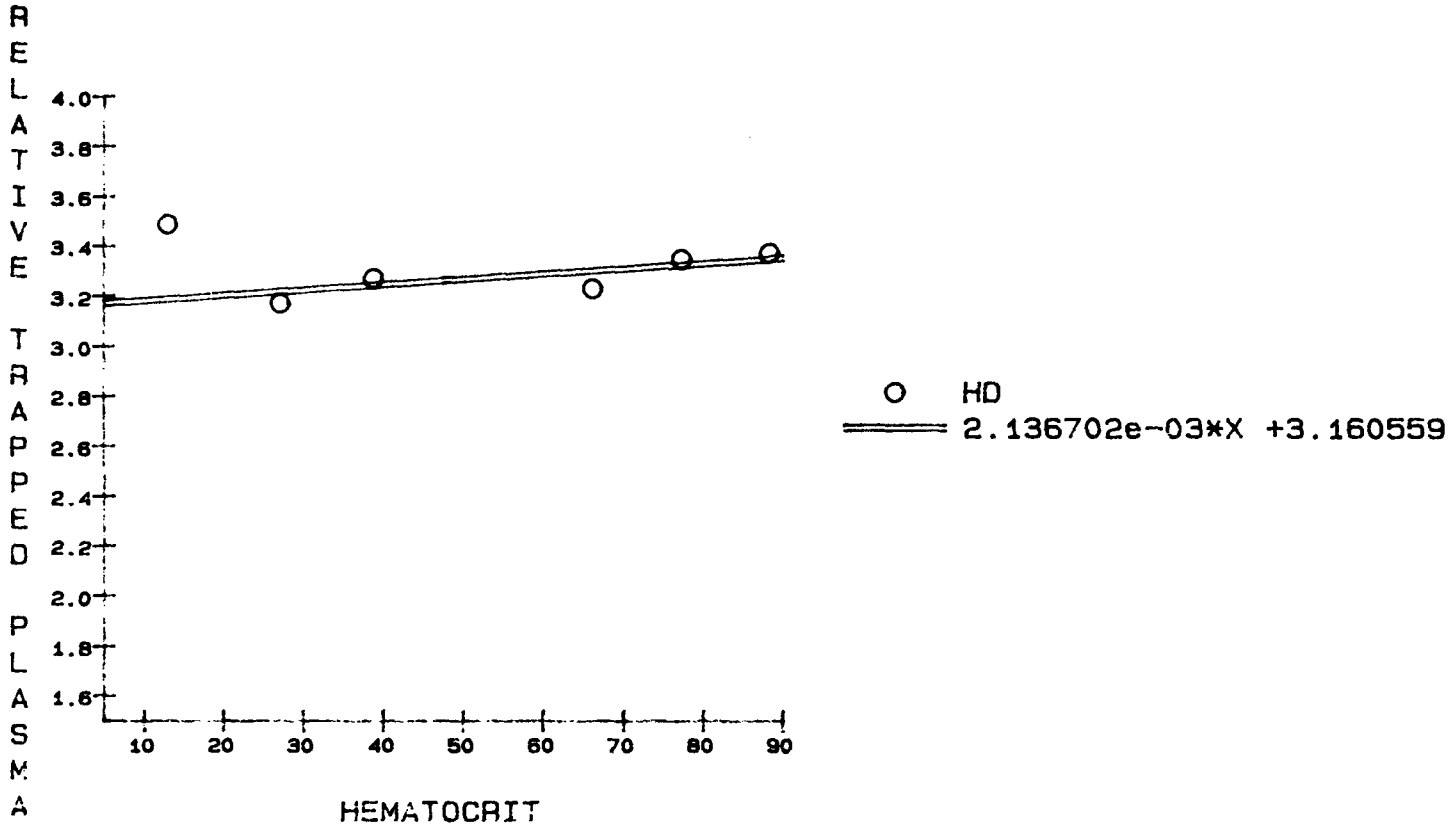


Figure 17. Hematocrit Level Effect (HD)

linear correlation is seen in all ten specimens, but the degree of slope varies somewhat from subject to subject.

Relative trapped plasma values actually increased in samples prepared with hematocrits of 15% or less. I am unable to explain this finding. All aspects of the techniques relating to the study were re-examined and no experimental flaw was uncovered. One potential experimental error is probably excluded on the basis of previously discussed studies, i.e. plasma segments greater than the critical 44 mm. Plasma did drain down into the base of the gamma counter tube. I have shown that the inner wall of the capillary tube does not retain ^{131}I counts which would be lost above the crystal during counting (See above Gamma Counting).

A second possible explanation, for the higher relative trapped plasma values, is that in extremely low hematocrits (<15%) the average cell has further to travel during centrifugation to achieve equivalent packing as compared to a specimen with a normal hematocrit. This greater distance may require a longer optimum packing time to achieve maximum packing than previously determined for samples with higher hematocrits. To evaluate whether a longer packing time is needed, five specimens were altered to yield hematocrits below 15%. Separate capillary tubes from each specimen were centrifuged for five and ten minutes.

Relative Trapped Plasma (%)

Patient	5 Min	10 Min	Difference
JS	2.0	1.9	-.1
BM	2.5	3.1	+.6
TP	2.4	1.8	-.6
JL	2.6	2.0	-.6
HD	3.7	3.4	-.3
Ave.	2.6	2.4	-.2

The relative trapped plasma at 10 minutes is slightly lower than at five minutes, but the differences are small, inconsistent, and probably within experimental error. In any case, they cannot explain the differences noted in the hematocrit alteration experiments.

Automated versus Manual Hematocrits

Fairbanks in 1980 (18) reported that centrifuged hematocrits are equivalent to hematocrits derived from electronic particle counters only within a specified range. He stated the two methods are equivalent only in hematocrits from 25% to 50%. Below 25% the centrifuged

hematocrit will be lower, and above 50% the centrifuged hematocrit will be higher than hematocrits from automated methods. He concludes, "This variation may be due to the tighter packing of the erythrocytes when the centrifuged hematocrit value was low and the looser packing of erythrocytes when the hematocrit value was high."

One would expect that an increase in the volume of trapped plasma would result in higher values for centrifuged hematocrits. As noted previously, (See above Effect of Hematocrit Level) this deviation between methods should increase with higher hematocrit levels because the g force acting on the column of blood is less at the upper end of the red cell column. This effect should be observed throughout the hematocrit scale. Electronic instruments are calibrated against reference material with centrifuged hematocrit values. These reference samples are usually taken from healthy volunteers with hematocrit values in the reference range. Hematocrit values on samples with low hematocrits should be lower than those from electronic instruments because the relative trapped plasma would be expected to be less.

To examine the validity of these assumptions, I obtained specimens from six healthy volunteers. Each specimen was divided into five aliquots. Serial dilutions of red cells were made using their respective plasmas, creating five aliquots of varying hematocrit within each

specimen. Hematocrit determinations were then made from each aliquot by the centrifugation method and by the electronic method (Coulter S Plus IV). The results of these studies are shown in Figure 18. When the results from these two hematocrit determination methods were plotted against each other, the data points fell on a line of identity in low and midrange hematocrit levels. At higher hematocrit levels, the centrifuged hematocrit value is larger than its automated counterpart. From this study, we conclude that the amount of trapped plasma is significant enough to inflate the centrifuged hematocrit value only at high hematocrit levels.

We are unable to ascertain deviations of microhematocrits from automated particle counter hematocrits at low hematocrit levels. However, the deviation noted by Fairbanks (10) at low hematocrit levels was quite small. Even if no plasma were trapped, the differences in hematocrits by the two methods would be miniscule (e.g. a change from 3% to 0% trapped plasma at a hematocrit of 10% would produce a hematocrit difference of only 0.3 hematocrit percent).

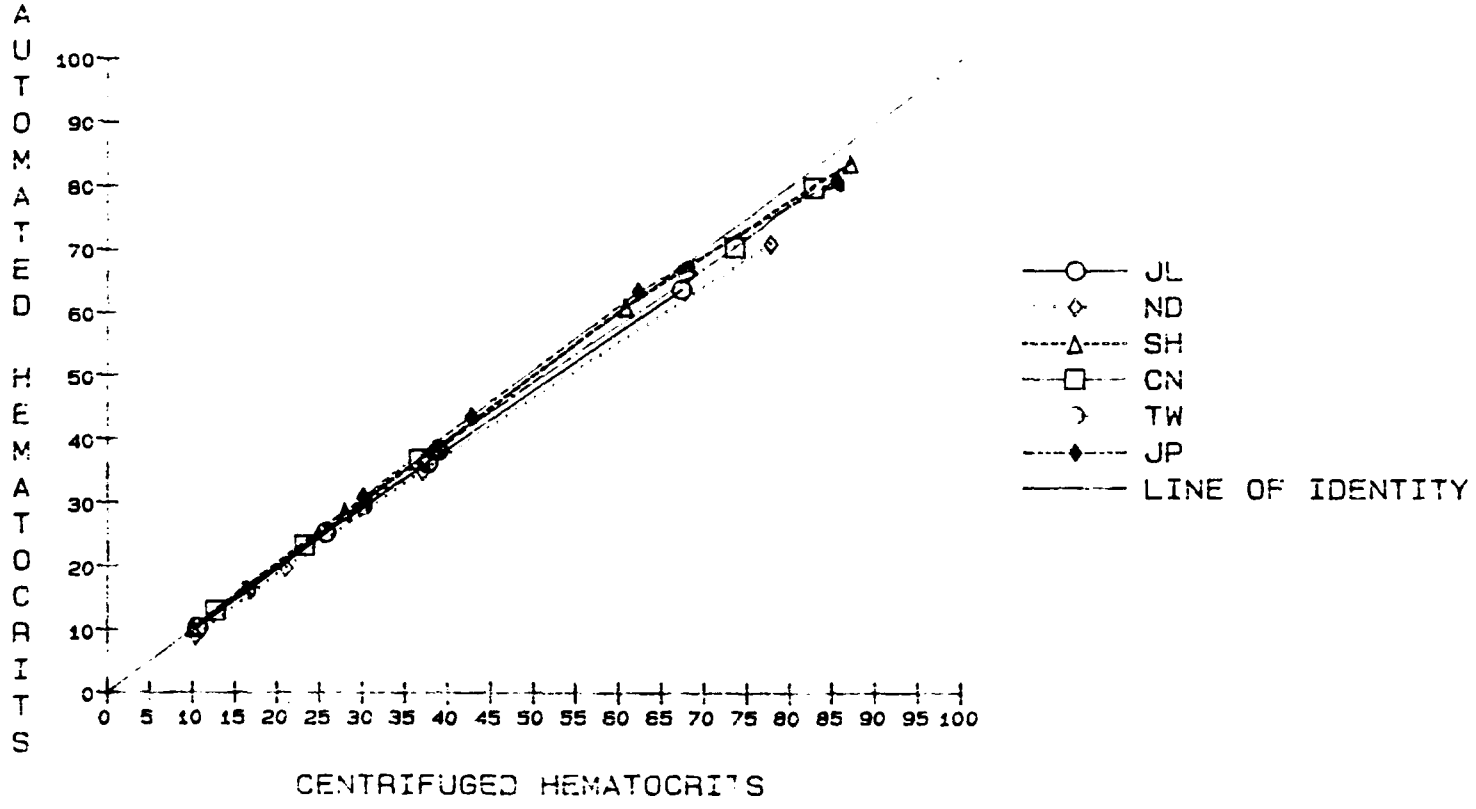


Figure 18. Hematocrit Level Effect

Relative Trapped Plasma Values of Patient Samples

Reference Range

Relative trapped plasma values were determined from samples of twenty-five healthy volunteers. The relative trapped plasma sample mean is 2.7%, with a highest determined value of 3.4% and lowest of 2.2%. In comparison, mean values for trapped plasma in previous studies (2,5-7) ranged from 1.3% to 3.2%. The mean, median, and mode are all 2.7%. There are not enough determinations to establish firmly whether the values are normally distributed. A Wilk-Shapiro Test of Normality on the values shows insufficient reason to reject the null hypothesis that the population is normal (See Figure 19). The distribution exhibits a small amount of negative skewness (-0.03) and deviates only slightly from the peakedness of a normal distribution as demonstrated by the measure of kurtosis (-0.58). Assuming a normal distribution, the 95% confidence limits ($\pm$2 SD) for relative trapped plasma in samples from healthy individuals is 2.0% to 3.4%.

Our reference range is similar to the ranges reported by the previous investigators (See page 6). Chaplin and Mollison's (5) data agree closely with ours. This is somewhat surprising since the centrifugal forces

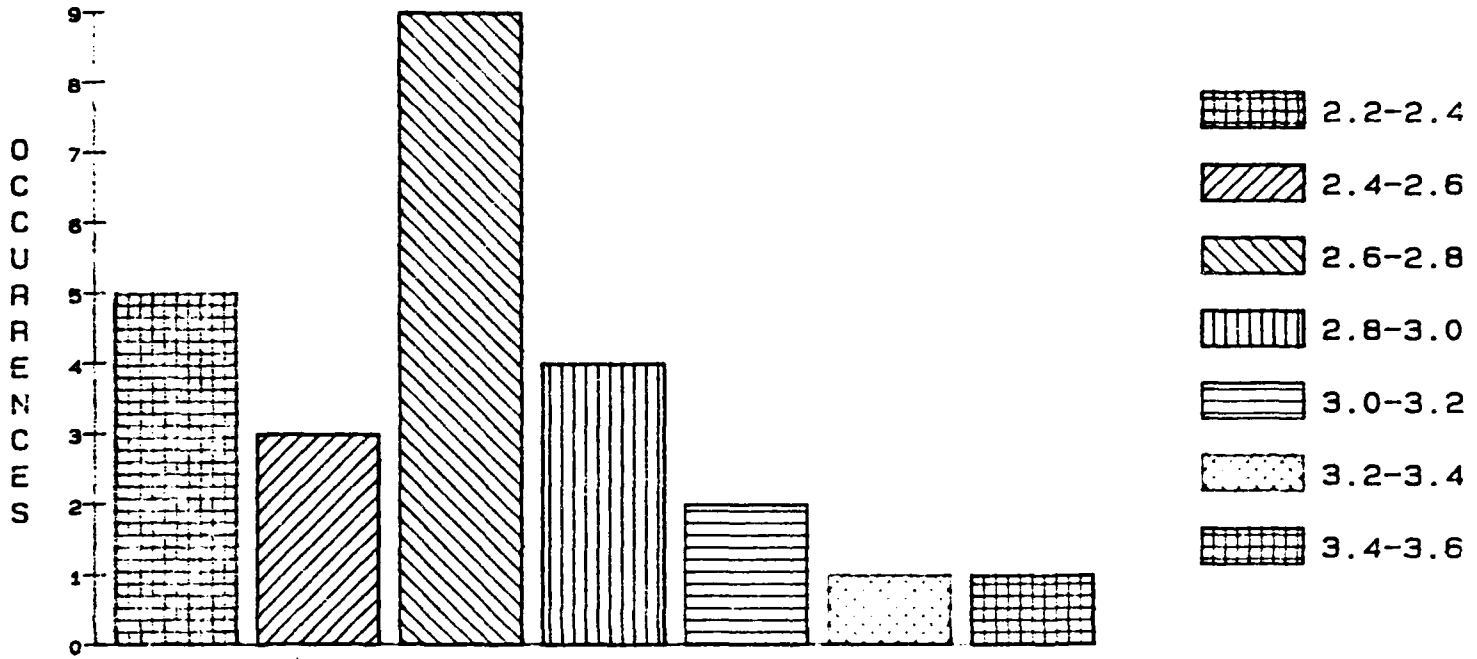


Figure 19. Reference Range Distribution

generated in their method were much lower than ours, resulting in hematocrits that are higher than that produced by the high speed centrifugation we used. The reference range established by Garby and Vuille (6) is expected to be somewhat lower than our range because their calculation of relative trapped plasma was based on a slightly different formula. The basis for this is not explained in their paper. The nominally higher values found by England, Walford, and Waters (2) are difficult to explain since their centrifugation method produced the highest centrifugal forces of any methods discussed.

The Day-to-Day Variability of Relative Trapped Plasma

To demonstrate the stability of the relative trapped plasma value day-to-day *in vivo*, separate samples from two volunteers were collected and assayed immediately at selected intervals over a period of several weeks. The specimens were from a healthy donor (TA) and an iron deficient patient (SJ). Hematocrit determinations and relative trapped plasma values were determined. The findings are:

Day-to-Day Variability

TA	DATE	10-24	11-5	11-8	11-25	3-4	MEAN
	TP	2.7%	2.7%	2.6%	3.1%	2.6%	2.7%
	HCT	43.0	42.5	43.2	44.2	44.6	43.5

SJ	DATE	2-3	2-18	2-26	3-4	MEAN
	TP	2.9%	2.4%	2.6%	2.9%	2.7%
	HCT	35.0	34.1	36.6	34.6	35.1

The degree of variability is well within the standard deviation of the method ($\pm$ 0.37, See above Standard Deviation of the Method).

We are unable to find mention of day-to-day variability in any of the previous studies.

Relative Trapped Plasma Values in Red Cell Disorders

We investigated relative trapped plasma values in four different categories of red cell disorders. The four categories are: 1) Microcytic cells - defined as cells with an MCV <78 fL, 2) Macrocytic cells - defined as cells with an MCV >98 fL, 3) Normocytic cells with shape abnormalities - cells with an MCV >78 fL, <98 fL, and

having moderate to marked poikilocytosis on the red cell morphology portion of the CBC, and 4) Sickle cell disease samples.

Microcytic Cells

Twenty-five patients with microcytic cells were evaluated. All had an MCV <78 fL. The data from these patients is presented in Table 4. The mean value for relative trapped plasma for all twenty-five samples is 2.7%. The microcytic samples were further subgrouped into three categories: Iron Deficient Anemia, Thalassemia, and Otherwise Undefined.

The criteria for the iron deficient subgroup was (1) cells from patients with an MCV <78 fL as determined by Coulter S Plus IV and/or (2) at least one of the following:

A.	Ferritin	<20 ng/mL
B.	Saturation of the TIBC	≤16%
C.	Protoporphyrin	>105 umol/mol heme

Two exceptions to the MCV criterion are the last two patients (KN and GC). Although their MCVs were above 78 fL, their iron studies clearly indicated that they are

TABLE 4:
MICROCYTIC CELLS

PAT	TP	HCT	MCV	MCH	MCHC	RDW	NOTE
IRON DEFICIENT ANEMIA:							
BK	2.43	37.3	64.5	21.2	32.9	22.5	Fe def (T)
SJ	2.89	35.0	67.9	21.1	31.0	20.4	
AM	2.26	38.5	75.0	24.8	33.1	19.8	
MB	3.86	35.4	75.4	24.9	33.1	22.4	
HH	1.88	26.3	76.9	24.9	32.4	18.9	
KN#	3.75	32.4	78.7	26.4	33.6	23.0	
GC#	2.68	26.1	84.1	29.4	35.0	19.9	MOD ELLIP
THALASSEMIA:							
EG	2.42	35.4	63.8	20.6	32.3	15.1	Beta
DP	2.86	41.5	65.3	20.7	31.7	16.4	Alpha
EK	2.78	39.6	67.2	22.0	32.8	15.0	Beta
JW	2.47	30.8	72.2	23.4	32.4	16.5	Sickle/ Beta Thalassemia
JD	2.30	41.8	76.1	24.8	32.6	13.4	Alpha
DIAGNOSIS UNKNOWN:							
GL	2.22	32.5	66.7	21.3	32.0	17.5	
CD	4.25	33.4	68.3	22.3	32.6	20.2	
RC	2.87	40.0	70.8	22.5	31.7	16.7	
DT	3.05	34.9	74.8	24.2	32.3	18.5	
MM	2.33	35.8	74.8	24.9	33.3	13.6	
KC	2.42	36.2	75.5	25.1	33.2	15.1	
ML	2.70	45.4	76.2	25.3	33.3	13.8	
LJ	2.25	25.5	76.4	25.9	33.9	13.7	
LJ	2.46	38.4	76.5	24.5	32.1	14.1	
FS	2.47	36.5	76.7	24.8	32.3	22.4	
JP	2.42	40.3	77.3	25.9	33.5	15.9	
SD	2.15	34.2	77.6	26.0	33.5	17.7	
KJ	2.20	45.0	77.8	25.1	32.2	13.4	

#Two patients whose cells did not meet normocytic criteria have been included here because they were found to have incipient microcytosis due to iron deficiency.
(T) Transfusion

iron deficient.

The mean value for the relative trapped plasma in samples from the seven iron deficient anemia patients is 2.8%.

The mean value for relative trapped plasma for the five thalassemia samples is 2.6%. The UW Hemolysis Lab confirmed these specimens as thalassemic using hemoglobin electrophoresis, quantitation of hemoglobin A_2, and/or demonstrating hemoglobin H containing red cells. Note that patient JW, who has sickle beta thalassemia, is included in data sets for both these disorders.

The remaining thirteen specimens are listed under the Diagnosis Unknown subgroup. Their mean value for relative trapped plasma value is 2.6%. These specimens did not meet the iron deficient anemia criteria, had normal hemoglobin fractions, or had microcytosis which was inadequately studied to exclude iron deficiency or thalassemia trait.

Macrocytic Cells

Thirteen samples with macrocytic cells were evaluated (See Table 5). The mean relative trapped plasma value is 3.0%. All of these specimens had an MCV > 98 fL. Within this macrocytic category, all but two patients fell into two major subgroups: alcoholism and diagnosis

TABLE 5:
MACROCYTIC CELLS

PAT	TP	HCT	MCV	MCH	MCHC	RDW
ALCOHOLISM:						
CB	2.28	27.7	108.6	37.1	34.1	13.5
EH	3.06	27.3	106.5	35.8	33.6	17.1
BM	3.20	29.0	103.8	36.2	34.9	15.9
JD	3.47	34.5	100.5	35.0	34.9	11.7
EM	2.41	35.8	98.1	32.5	33.1	13.4
B12/FOLATE DEFICIENCY:						
ID	2.23	29.1	120.4	41.5	34.5	17.7
PNH:						
BD	2.28	19.3	115.2	38.4	33.3	17.5
DIAGNOSIS UNKNOWN:						
LB	3.26	34.0	105.1	36.4	34.6	16.1
CF	2.26	32.6	103.5	34.7	33.5	12.2
MK	2.11	40.0	100.7	34.8	34.5	12.5
JC	3.85	28.1	100.6	35.5	35.3	15.2
LB	3.73	32.2	100.1	35.0	34.9	13.3
RE	5.02	34.0	100.1	33.6	33.6	14.3

unknown. One patient had B_{12} deficiency and one had paroxysmal nocturnal hemoglobinuria.

The five samples from patients in the alcoholism subgroup had a mean relative trapped plasma of 2.9%. These patients had chronic abuse of alcohol documented in their medical records.

The six samples in the diagnosis unknown subgroup had a mean relative trapped plasma of 3.4%. In this subgroup, patient RE had the highest determined relative trapped plasma in the entire project.

Normocytic Cells with Shape Abnormalities

The ten normocytic cell samples with shape abnormalities had a mean relative trapped plasma of 2.8% (See Table 6). These ten samples had red cells that were normocytic but were indicated as having marked or moderate poikilocytosis in the red cell morphology section of their CBC. One exception to this criterion is patient LJ who had a well-documented case of polycythemia vera.

Sickle Cell Disease

The five samples from sickle cell disease patients had a mean trapped plasma value of 2.4% (See Table 7). The designation of sickle cell disease was confirmed by

TABLE 6:
NORMOCYTIC CELLS WITH SHAPE ABNORMALITIES

PAT	TP	HCT	MCV	MCH	MCHC	RDW	NOTATION ON SMEAR
EN	1.92	30.6	91.5	30.4	33.3	21.2	*MOD ELLIPTOCYTOSIS
VW	2.28	42.5	90.7	30.4	33.5	17.0	*MOD ELLIPTOCYTOSIS
JW	4.86	35.0	90.4	31.0	34.3	13.5	*MOD ELLIPTOCYTOSIS
CD	3.74	46.6	88.3	29.6	33.5	19.3	*MOD TARGETS
LF	2.50	31.9	83.8	28.1	33.5	13.8	*MOD ELLIPTOCYTOSIS
SP	2.85	30.3	83.6	32.5	38.9	27.8	*MOD SPHEROCYTOSIS
AN	2.09	35.2	82.7	26.5	32.1	15.6	*MOD ELLIPTOCYTOSIS
JW	2.64	40.2	81.5	26.6	32.6	14.3	*MOD ELLIPTOCYTOSIS
GJ**	2.37	37.3	79.9	26.8	33.6	14.2	#MAR ACANTHOCYTOSIS
LJ**	2.72	56.3	78.4	24.3	30.9	27.4	P VERA

*MODERATE
#MARKED
**INCLUDED HERE BECAUSE SAMPLES DID NOT MEED OUR MICROCYTOSIS CRITERIA.

TABLE 7:
SICKLE CELL DISEASE

PAT	TP	HCT	MCV	MCH	MCHC	RDW	NOTE
JW	2.47	30.8	72.2	23.4	32.4	16.5	Sickle/ Beta Thalassemia
MW	2.79	24.5	74.0	23.9	32.3	23.6	Sickle cell anemia
AN	2.85	30.5	88.0	29.5	33.5	15.0	HbSC disease*
AE	2.11	27.8	90.1	30.3	33.6	15.2	Sickle cell anemia (T)
MD	1.92	21.9	102.7	34.4	33.5	14.1	Sickle cell anemia

(T) Transfusion
*sample processed 18 hours after collection

the UW Hemolysis Lab. Sickle cell screens were performed using hemoglobin electrophoresis (cellulose acetate) and other tests as appropriate to confirm the diagnosis. Note that patient AE had a recent transfusion prior to evaluation. Also note that the value for patient AN was determined after approximately 18 hours incubation. The effect of incubation is discussed above.

Comparison of Data on Disease States from Previous Studies

Our data show little variation in trapped plasma values in samples from patients with a variety of red cell diseases. This result contrasts with reports by previous investigators. Chaplin and Mollison reported increased relative trapped plasma values in one case of iron deficient anemia and one case of polycythemia vera. Their general conclusion, based on a limited number of diseased subjects, is that increased plasma trapping is related to lower red cell size. They stated that anisocytosis and spherocytosis by themselves do not alter the amount of trapped plasma, and that microcytic cells exhibit significantly higher trapped plasma amounts than macrocytic cells. England, et al demonstrated higher trapped plasma values in one sample from sickle cell trait and in several samples from patients with hypochromic anemia and macrocytic anemia. Pearson and Guthrie

examined a variety of red cell disorders. However, they were only able to discern statistically higher trapped plasma values in samples from iron deficient anemia and homozygous Hb S patients.

Rosa, et al (18) are the only other investigators we found who had studied sickle cell disease patients. They state that trapped plasma in sickle cell disease patients' red cells is negligible. No actual data were given.

It is difficult to speculate on the reasons for such wide ranging trapped plasma values in red cell diseases. We do not believe that the methodology differences between studies that can explain the differences in the results.

The Relative Trapped Plasma Value Correlation to RDW

Newer electronic cell counters provide a measurement called the red cell distribution width (RDW). This measurement represents an integrated value for the frequency distribution of the red cell sizes in a blood sample, essentially a quantitative measure of anisocytosis. It is claimed to provide a new diagnostic tool for disorders of erythropoiesis. We were interested in studying trapped plasma in a variety of these disorders and comparing the results of these two techniques.

All of the specimens with red cell disorders had been previously assayed on a Coulter S Plus IV as part of

their initial patient workup. This analysis automatically includes the RDW.

When the trapped plasma values of all fifty-two specimens were compared to their RDW values, little correlation was observed (See Figure 20, $p > .25$, $r = .07$). When the relative trapped plasma values of only the twenty-three patients with microcytic cell disorders were compared to their respective RDW values, the correlation is more evident (See Figure 21, $.005 < p < .01$, $r = .48$).

This correlation is interesting and one might speculate that it relates to known fragmentation which occurs with increasingly microcytic cells, both in iron deficiency and in thalassemia. Perhaps these fragments are also more rigid and pack less efficiently. Another speculation (not borne out by our observations) might be that the increasingly small microcytes would actually fit together more compactly and trapped plasma would be decreased.

The comparisons of RDW values to relative trapped plasma values in the other three major categories (macrocytic cells, normocytic cells with shape abnormalities, and sickle cell disease) are not significant as demonstrated by $p > .25$ in all three cases (See Figures 22-24).

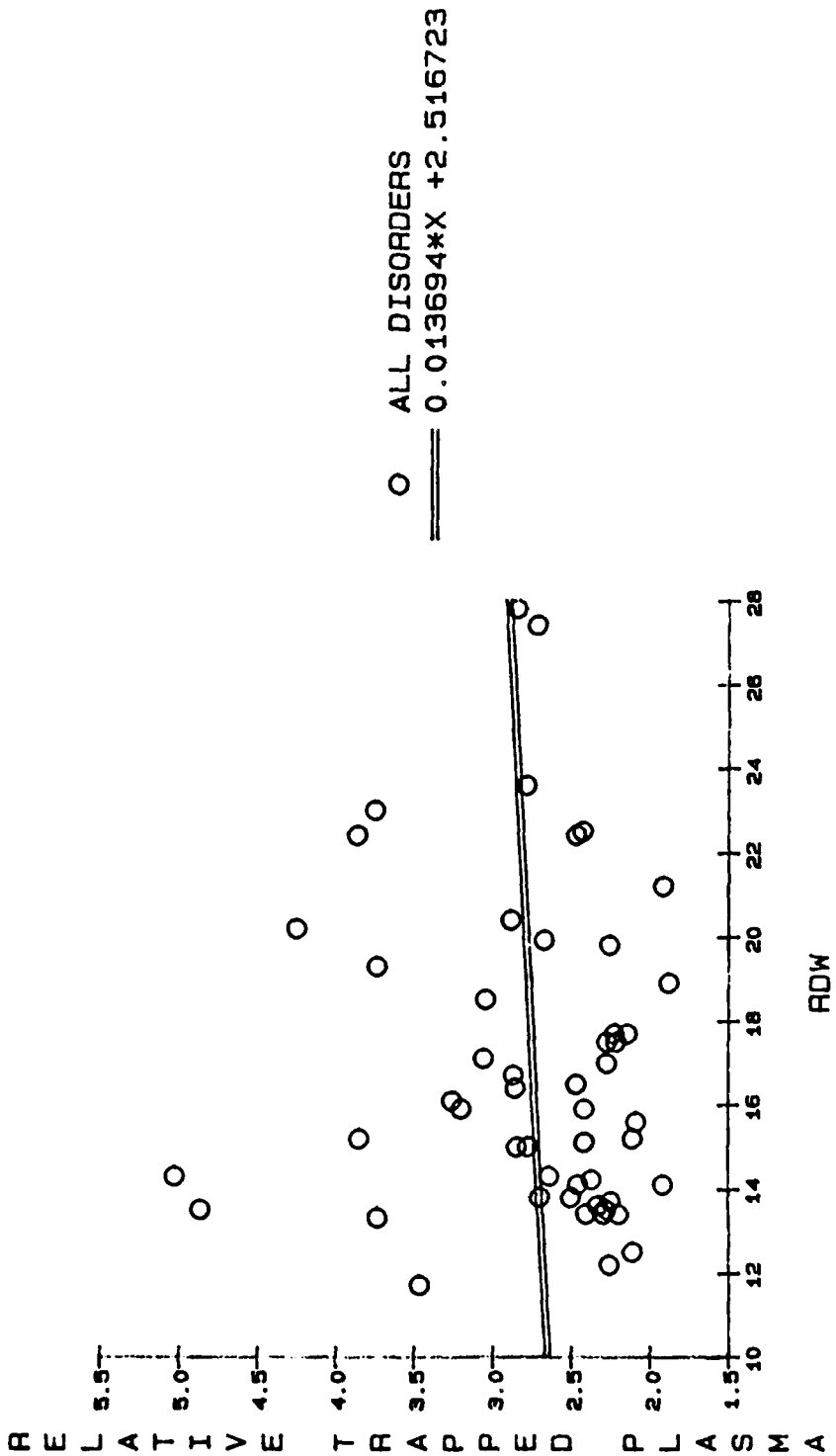


Figure 20. All Red Cell Disorders

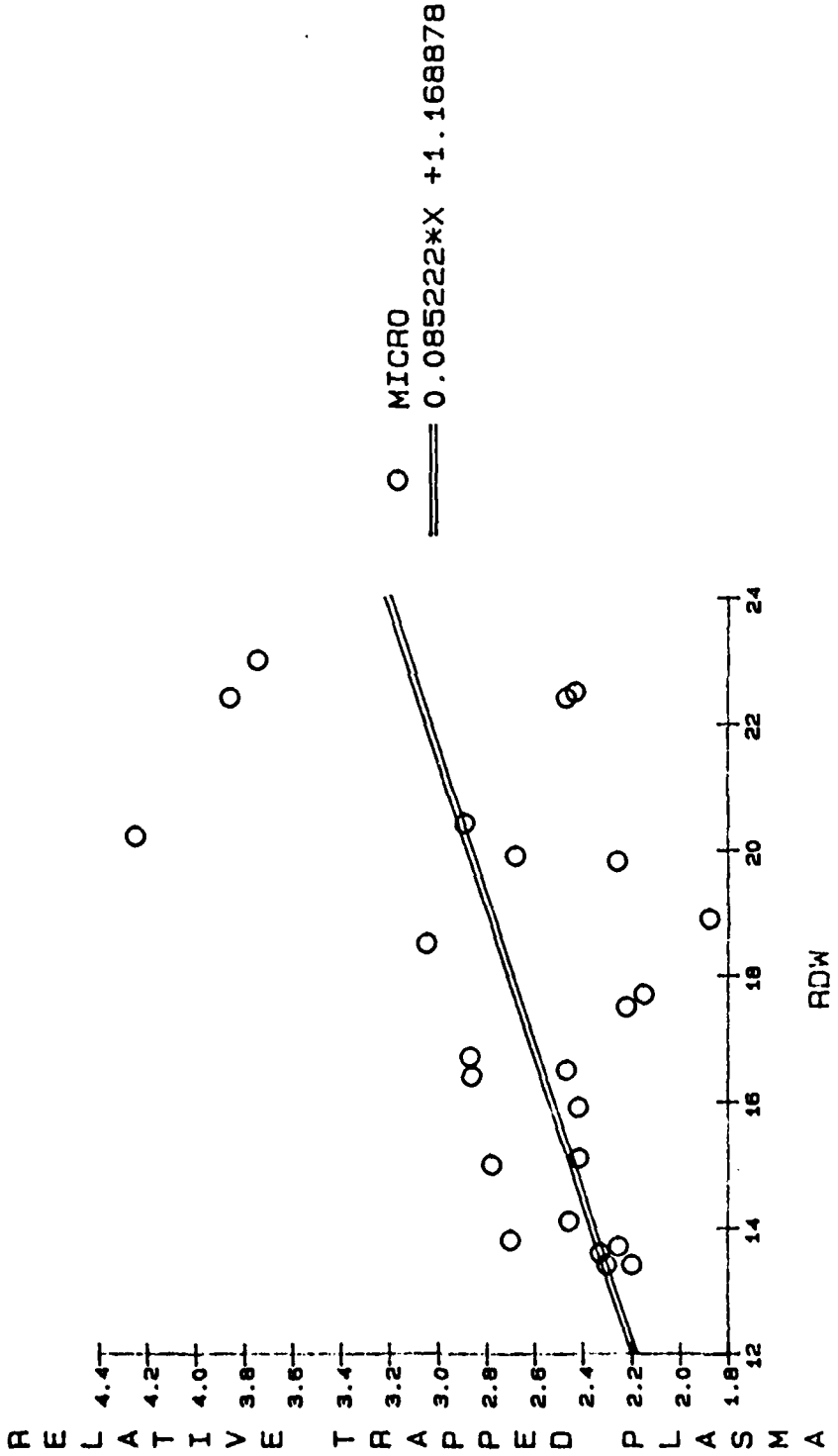


Figure 21. Microcytic Cells

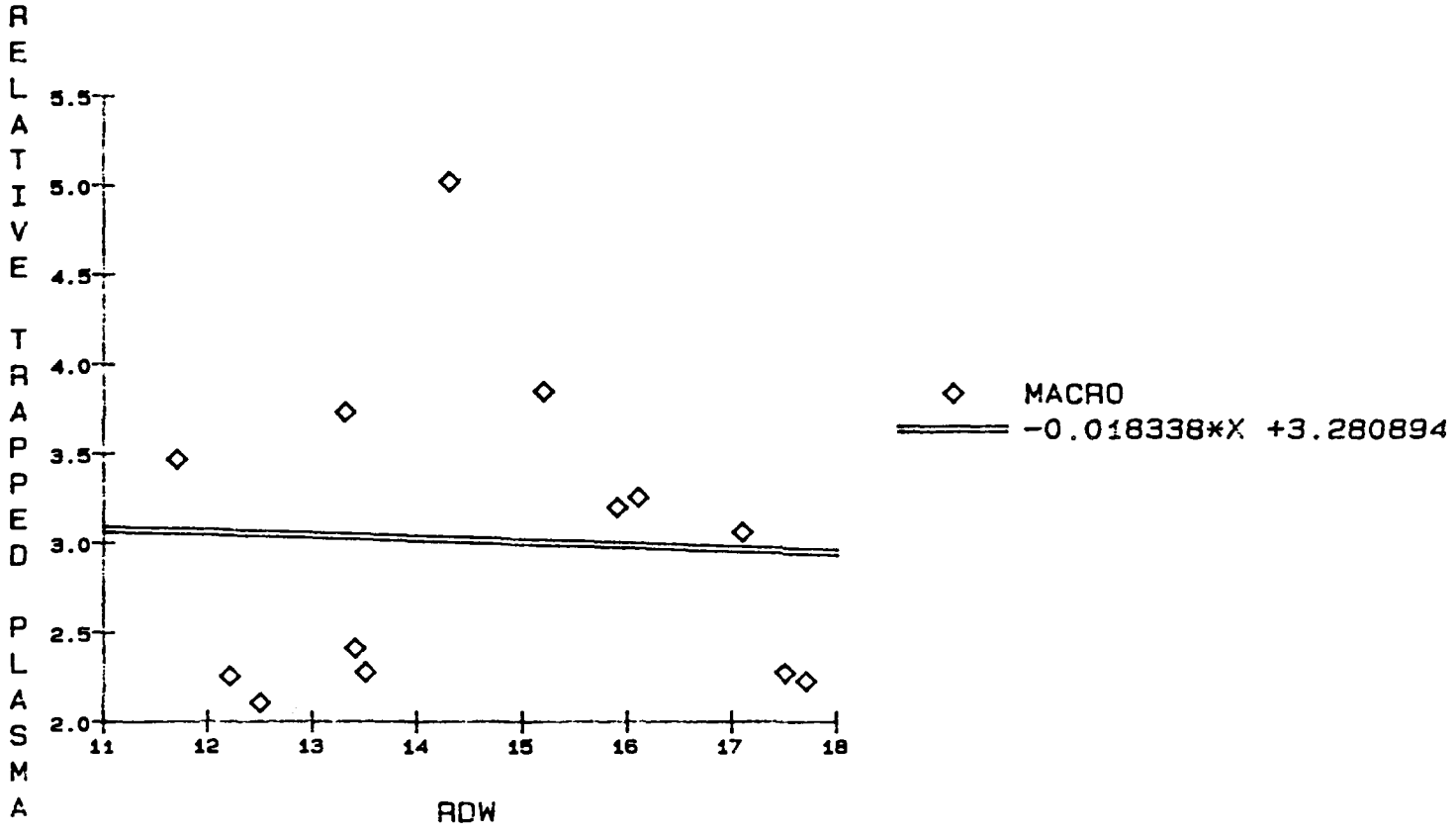


Figure 22. Macrocytic Cells

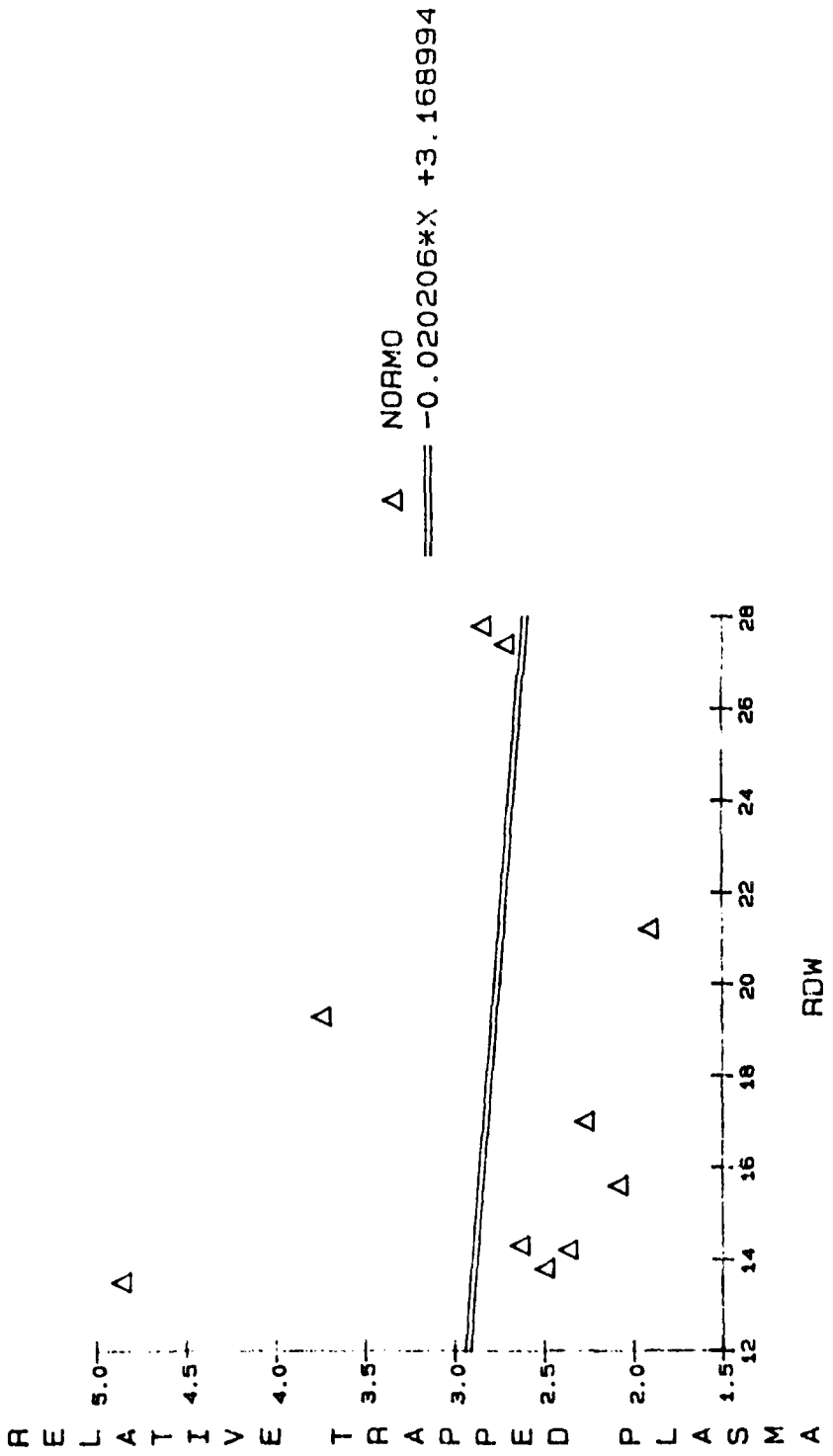


Figure 23. Normocytic Cells with Shape Abnormalities

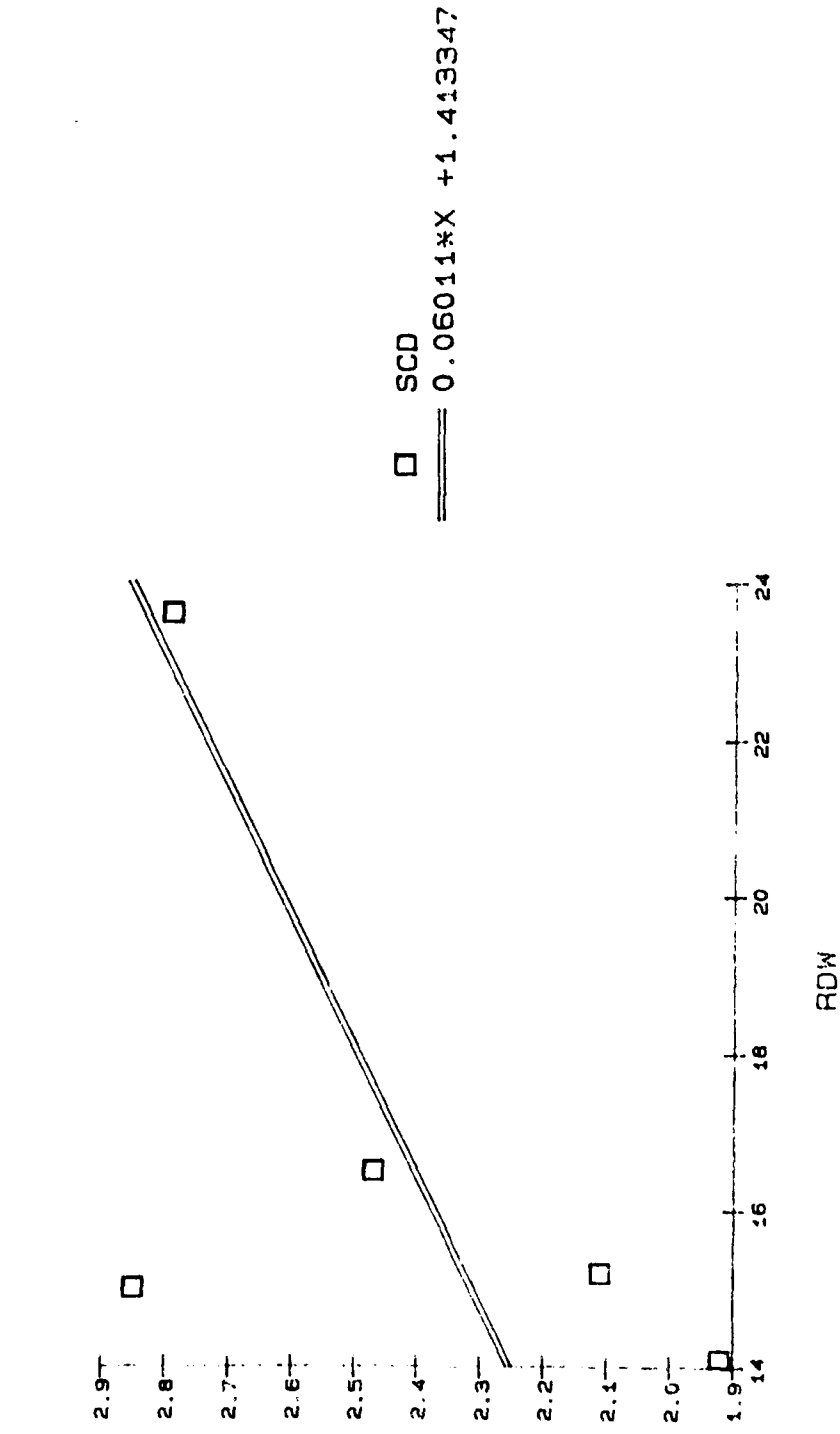


Figure 24. Sickle Cell Disease

CHAPTER FOUR

CONCLUSION

Conclusions

As a result of these studies, we propose the following:

1. Monitoring hematocrit instrument performance by periodic measurement of optimal packing:

Based on our data, we believe it is unnecessary to check constant packing of cells at specific time intervals for the microhematocrit method. We do not feel that this practice, which is mandated by many certification agencies, can be defended by scientific evidence. Our data indicate that constant packing is achieved in five minutes at centrifuge speeds attained by centrifuges used in most American medical laboratories. A check of centrifuge speed with a stroboscope and a check of accuracy of the centrifuge timer at regular monthly intervals are the only tests that are needed. As long as the centrifuge maintains the prescribed speed and an adequate packing time is used, the additional packing beyond five minutes will be miniscule.

2. Large increases in trapped plasma in association with red cell diseases are not found using our hematocrit methodology:

Previous suggestions that there are marked

variations in relative trapped plasma values in certain red cell diseases are not borne out by our study. Our data suggests that the amount of trapped plasma in selected red cell disorders is not statistically different from the reference value. However, we did detect a noticeable difference in trapped plasma when the hematocrit level was markedly increased. This increased amount of trapped plasma is a reflection of decreased gravitational forces at the top end of the packed cell column, and therefore is seen in diseased, as well as in healthy blood.

Advancing the above conclusion one step further, the effect of trapped plasma on the hematocrit level of diseased blood will be even smaller than on samples from healthy subjects because the hematocrit levels from patients with these selected red cell disorders are usually lower.

3. Correction of the hematocrit for trapped plasma:

We do not believe that the hematocrit value should be corrected for the trapped plasma error. First, the amount of trapped plasma varies with the hematocrit level of the sample so that a standard correction factor is not possible. Secondly, the difference caused by trapped plasma in hematocrits of reference range is very small. Finally, if a trapped plasma correction factor were agreed upon, a certain degree of confusion might ensue because

some labs would choose not to follow this correction policy.

We do support Fairbanks' conclusion that patients with hematocrits >50% should be followed using an automated particle counter which calculates the hematocrit from red cell count and cell size. This will avoid the problem of significant inflation of the hematocrit level introduced by trapped plasma at high hematocrit levels.

4. Reliability of the mean corpuscular hemoglobin concentration (MCHC):

Recently, there has been an argument as to whether the MCHC derived from automated particle counters is reliable. This is based, in part, on discrepancies between the counters which use a hematocrit calculated from the red count and cell size in the MCHC calculation and the MCHC calculation using the centrifuge derived hematocrit. This difference is particularly evident in subjects with iron deficiency. The MCHCs in these subjects are often normal from automated instruments while the MCHC calculated from the centrifuged hematocrit is low. Another explanation for the differences between the two methods might be that the centrifuged value is erroneously low due to increased trapped plasma in iron deficient centrifuged samples. We are unable to demonstrate increased trapped plasma in our iron deficient patient samples. However, our sample size is small and

our patients were not severely deficient (as manifested by severe anemia). An increased amount of trapped plasma might be more apparent in more severely deficient patients.

Suggested Prospective Studies

We suggest the following studies on trapped plasma for future investigations:

1. The effect of plasma osmolality on trapped plasma:

We found one study by Morris and Reece (1983) that was carried out on canine blood. Their conclusion is that the plasma osmolality would have to be tripled before any noticeable change in trapped plasma occurs. We believe this work should be duplicated on human blood using salts seen in osmolality disorders.

2. The effect on trapped plasma in more severe red cell disorders:

Isolated clinical observations of striking disparities between microhematocrit and automated hematocrit methods have been observed. Examples where disparities have been observed include: samples from patients with severe hemoglobinization defects such as thalassemia major and sideroblastic anemia. Additionally, hereditary spherocytosis should be evaluated since the spherocyte has the maximum volume to surface area ratio.

This geometric shape alone might result in higher relative trapped plasma values since these cells would be expected to be less deformable. Finally, lipid disorders associated with changes in membrane lipids might show abnormalities in deformability. Examples of disorders associated with abnormal membrane lipids include hypercholesterolemia, biliary cirrhosis, and the hereditary and acquired forms of acanthocytosis.

3. The effect on trapped plasma in blood with excessive protein:

Large amounts of protein in blood might also cause an elevation in the amount of trapped plasma. Examples are patients with multiple myeloma and cord blood from the newborn.

LIST OF REFERENCES:

1. Morris TW, Reece K. Trapped plasma vs. osmolality in canine microcapillary hematocrits. Invest Rad 1983; 18(6):523-529.

2. England JM, Walford DM, Waters DAW. Re-assessment of the reliability of the hematocrit. Br J Haematol 1972; 23:247-256.

3. Schwartz E, Gill FM. Ch. 5, Hematology of the newborn, in Williams WJ, Beutler E, Ersler AJ, Lichtman MA. Hematology, 3ed, McGraw-Hill, 1983; p. 38.

4. Penn D, Williams PR, Dutcher TF, Adair RM. Comparison of hematocrit determinations by microhematocrit and electronic particle counter. Am J Clin Pathol 1978; 72:71-74.

5. Chaplin H, Mollison PL. Correction for plasma trapped in the red cell column of the hematocrit. Blood 1952; 7:1227-1238.

6. Garby L, Vuille JC. The amount of trapped plasma in a high speed micro-capillary hematocrit centrifuge. Scan J Clin Lab Invest 1961; 13:642-645.

7. Pearson TC, Guthrie DL. Trapped plasma in the microhematocrit. Am J Clin Pathol 1982; 78:770-772.

8. Furth FW. Effect of spherocytosis on volume of trapped plasma in red cell column of capillary and Wintrobe hematocrits. J Lab Clin Med 1956; 48(3):421-429.

9. Economou-Mavrou C, Tsenghi C. Plasma trapping in the centrifuged red cells of children with severe thalassemia. J Clin Path 1965; 18:203-208.

10. Fairbanks VF. Nonequivalence of automated and manual hematocrit and erythrocyte indices. Am J Clin Pathol 1980; 73:55-62.

11. Henry JB. Clinical diagnosis and management by laboratory methods. 16th ed. W.B. Saunders Co., Philadelphia PA 1979; p. 872

12. England JM, Koedam JC, Archer RK, Crosland-Taylor P, Helleman PW,Lewis SM, Thom R. Recommendation for reference method for determination by centrifugation of packed cell volume of blood. J Clin Pathol 1980; 33:1-2.

13. Miale JB. Laboratory medicine, hematology. 5th ed. The CV Mosby Co., St. Louis, Mo, 1977; p. 424.

14. Multi Prias 2 Operating Manual. United Technologies, Packard Industries.

15. Wang CH, Willis DL, Loveland WD. Radiotracer methodology in the biological, environmental, and physical sciences. McGraw-Hill Book Co., 1975; 302-305.

16. UW Radiation Safety Office. Principles of radiation protection. 1985; p. 28.

17. Lines RW, Grace E. Choice of anticoagulants for packed cell volume and mean cell volume determination. Clin Lab Haemat. 1984; 6:305-306.

18. Rosa RM, Bierber BE, Thomas R, et al. A study of induced hyponatremia in the prevention and treatment of sickle-cell disease. New Engl J Med 1980; 303(20): 1138-1143.

END

DTIC

9-86